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```
=> s (serum albumin)
        574652 SERUM
        101650 ALBUMIN
L1       68146 (SERUM ALBUMIN)
                (SERUM(W)ALBUMIN)

=> s l1 and (CYS-34 or cysteine 34)
         12759 CYS
        185429 34
            83 CYS-34
                (CYS(W)34)
         63438 CYSTEINE
        185429 34
            30 CYSTEINE 34
                (CYSTEINE(W)34)
L2          74 L1 AND (CYS-34 OR CYSTEINE 34)

=> s l2 and py<2000
      12397709 PY<2000
L3          39 L2 AND PY<2000

=> d l3,cbib,ab,1-39
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L3 ANSWER 1 OF 39 MEDLINE on STN
2000210399. PubMed ID: 10746304. Comparison of formats for the development of fiber-optic biosensors utilizing sol-gel derived materials entrapping fluorescently-labelled protein. Flora K; Brennan J D. (Department of Chemistry, McMaster University, Hamilton, Ontario, Canada.) Analyst, **(1999 Oct)** 124 (10) 1455-62. Journal code: 0372652. ISSN: 0003-2654. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The development of fiber-optic biosensors requires that a biorecognition element and a fluorescent reporter group be immobilized at or near the surface of an optical element such as a planar waveguide or optical fiber. In this study, we examined a model biorecognition element-reporter group couple consisting of human **serum albumin** that was site-selectively labelled at **Cys 34** with iodoacetoxy-nitrobenzoxadiazole (HSA-NBD). The labelled protein was encapsulated into sol-gel derived materials that were prepared either as monoliths, as beads that were formed at the distal tip of a fused silica optical fiber, or as thin films that were dipcast along the length of a glass slide or optical fiber. For fiber-based studies, the entrapped protein was excited using a helium-cadmium laser that was launched into a single optical fiber, and emission was separated from the incident radiation using a perforated mirror beam-splitter, and detected using a monochromator-photomultiplier tube assembly. Changes in fluorescence intensity were generated by denaturant-induced conformational changes in the protein or by iodide quenching. The analytical parameters of merit for the different encapsulation formats, including minimum protein loading level, response time and limit-of-detection, were examined, as were factors such as protein accessibility, leaching and photobleaching. Overall, the results indicated that both beads and films were suitable for biosensor development. In both formats, a substantial fraction of the entrapped protein remained accessible, and the entrapped protein retained a large degree of conformational flexibility. Thin films showed the most rapid response times, and provided good detection limits for a model analyte. However, the entrapment of proteins into beads at the distal tip of fibers provided better signal-to-noise and signal-to-background ratios, and required less protein for preparation. Hence, beads appear to be the most viable method for interfacing of proteins to optical fibers.

L3 ANSWER 2 OF 39 MEDLINE on STN
2000034160. PubMed ID: 10568165. Interaction of acrylodan with human **serum albumin**. A fluorescence spectroscopic study. Moreno F; Cortijo M; Gonzalez-Jimenez J. (Departamento de Quimica-Fisica Farmaceutica, Facultad de Farmacia U.C.M., Madrid, Spain.) Photochemistry and photobiology, **(1999 Nov)** 70 (5) 695-700. Journal code: 0376425. ISSN: 0031-8655. Pub. country: United States. Language: English.

AB The binding of the fluorescent probe acrylodan (AC) to human **serum albumin** (HSA) was studied by fluorescence spectroscopy. The binding

isotherms could be fitted to two types of sites. Competition experiments using iodoacetamide suggested that AC binds tightly on HSA by the **cysteine-34**. Attempts were made to find the location of the second site using high concentrations of warfarin, phenylbutazone, diazepam, indomethacin, palmitic acid or bilirubin in order to displace the bound AC to the HSA. Bilirubin was the only ligand able to displace the bound AC. This result suggests that AC, which is a very hydrophobic molecule also capable of labeling lysine residues, should also bind the human albumin in the primary site of bilirubin, but with less affinity than to the **cysteine-34**.

L3 ANSWER 3 OF 39 MEDLINE on STN
1999331485. PubMed ID: 10403053. Irreversible adsorption of human **serum albumin** to hydrogel contact lenses: a study using electron spin resonance spectroscopy. Garrett Q; Griesser H J; Milthorpe B K; Garrett R W. (Cooperative Research Centre for Eye Research and Technology, University of New South Wales, Sydney, Australia.. q.garrett@cclru.unsw.edu.au) . Biomaterials, **(1999 Jul)** 20 (14) 1345-56. Journal code: 8100316. ISSN: 0142-9612. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Human **serum albumin** (HSA) was specifically spin labelled with 4-maleimido-tempo (MSL) at its **cysteine 34** residue (HSA-MSL). The irreversible adsorption of HSA-MSL to hydrogel contact lenses (etafilcon A, tefilcon and vifilcon A) was investigated using electron spin resonance (ESR) spectroscopy. Changes in ESR spectral characteristics of adsorbed HSA-MSL as compared to HSA-MSL in solution displayed an additional immobilisation of the spin label due to the adsorption. This immobilisation of MSL corresponds to a large conformational alteration of the HSA-MSL near the modified **Cys 34** residue. For both etafilcon A and tefilcon, the rate of irreversible adsorption was relatively slow compared with that of vifilcon A where the maximum state of immobilisation and hence conformational change occurred within the first hour of adsorption. Furthermore, tefilcon produced markedly different ESR spectra where a strong conformational change to a less mobile protein was apparent. This supported a model where the direct irreversible adsorption of HSA from solution dominated on tefilcon as opposed to conversion of the adsorbed protein from the reversible to the irreversible state on both etafilcon A and vifilcon A. HSA-MSL adsorption onto hydrophobic poly(methylmethacrylate) (PMMA) and hydrophilic poly(N-ter-butylacrylamide) (PTBAM) latex beads was also investigated. The spin label MSL was found to be less mobile when HSA was adsorbed onto PMMA compared with PTBAM beads. It was also found that the rate of irreversible adsorption of HSA is far higher onto PMMA surfaces than onto PTBAM surfaces.

L3 ANSWER 4 OF 39 MEDLINE on STN
1999238388. PubMed ID: 10219106. Investigation of slow dynamics of the sulfhydryl in the solution and gel states of bovine **serum albumin**: A vector electron paramagnetic resonance study. Hayashi T; Shimoyama Y; Kuwata K; Era S. (Department of Physiology, Gifu University School of Medicine, Gifu, 500-8705, Japan.) Japanese journal of physiology, **(1999 Feb)** 49 (1) 27-33. Journal code: 2985184R. ISSN: 0021-521X. Pub. country: Japan. Language: English.

AB **Serum albumin** has one reactive sulfhydryl (**Cys-34**) that is one of the important binding sites. **Cys-34** is located in the crevice on the surface of the albumin molecule and is therefore restricted in its motion. Bovine **serum albumin** (BSA) Fraction V forms a transparent gel at pD 4.0 (F-form) in D2O at protein concentrations above 7% (BSA*-gel). We studied the molecular motion of **Cys-34** on BSA in the solution and gel states by the vector electron paramagnetic resonance (EPR) method using a maleimide spin label. The rotational correlation times of the spin label bound to **Cys-34** in the BSA solution and BSA*-gel were in the order of 10(-6) and 10(-5) s, respectively. A longer rotational correlation time of the **Cys-34** spin label in the BSA*-gel suggested that the gel network formed in BSA may drastically slow the motion of **Cys-34**. The integrated value obtained from the vector EPR spectra also showed an extremely dramatic slowing of the **Cys-34** spin label during the gel formation. On the other hand, the values for order parameter and the inclination of the principal axis (z) of the **Cys-34** spin label to the rotational axis (mu) were the same in the BSA solution and BSA*-gel.

L3 ANSWER 5 OF 39 MEDLINE on STN
1999091622. PubMed ID: 9873023. Kinetics of peroxynitrite reaction with amino acids and human **serum albumin**. Alvarez B; Ferrer-Sueta G; Freeman B A; Radi R. (Laboratorio de Enzimologia, Unidad Asociada Enzimologia, Universidad de la Republica, 11800 Montevideo, Uruguay.) Journal of biological chemistry, **(1999 Jan 8)** 274 (2) 842-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB An initial rate approach was used to study the reaction of peroxynitrite with human **serum albumin** (HSA) through stopped-flow spectrophotometry. At pH 7.4 and 37 degreesC, the second order rate constant for peroxynitrite reaction with HSA was 9.7 +/- 1.1 x 10(3) M-1 s-1. The rate constants for sulfhydryl-blocked HSA and for the single sulfhydryl were 5.9 +/- 0.3 and 3.8 +/- 0.8 x 10(3) M-1 s-1, respectively. The corresponding values for bovine **serum albumin** were also determined. The reactivity of sulfhydryl-blocked HSA increased at acidic pH, whereas plots of the rate constant with the sulfhydryl versus pH were bell-shaped. The kinetics of peroxynitrite reaction with all free L-amino acids were determined under pseudo-first order conditions. The most reactive amino acids were cysteine, methionine, and tryptophan. Histidine, leucine, and phenylalanine (and by extension tyrosine) did not affect peroxynitrite decay rate, whereas for the remaining amino acids plots of kobs versus concentration were hyperbolic. The sum of the contributions of the constituent amino acids of the protein to HSA reactivity was comparable to the experimentally determined rate constant, where cysteine and methionine (seven residues in 585) accounted for an estimated 65% of the reactivity. Nitration of aromatic amino acids occurred in HSA following peroxynitrite reaction, with nitration of sulfhydryl-blocked HSA 2-fold higher than native HSA. Carbon dioxide accelerated peroxynitrite decomposition, enhanced aromatic amino acid nitration, and partially inhibited sulfhydryl oxidation of HSA. Nitration in the presence of carbon dioxide increased when the sulfhydryl was blocked. Thus, **cysteine 34** was a preferential target of peroxynitrite both in the presence and in the absence of carbon dioxide.

L3 ANSWER 6 OF 39 MEDLINE on STN

1998278978. PubMed ID: 9614070. Cisplatin binding sites on human albumin. Ivanov A I; Christodoulou J; Parkinson J A; Barnham K J; Tucker A; Woodrow J; Sadler P J. (Department of Chemistry, University of Edinburgh, Edinburgh EH9 3JJ, United Kingdom.) Journal of biological chemistry, **(1998 Jun 12)** 273 (24) 14721-30. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Reactions of cisplatin (cis-[PtCl2(NH3)2]) with albumin are thought to play an important role in the metabolism of this anticancer drug. They are investigated here via (i) labeling of cisplatin with 15N and use of two-dimensional 1H,15N NMR spectroscopy, (ii) comparison of natural human **serum albumin** with recombinant human albumin (higher homogeneity and SH content), (iii) chemical modification of Cys, Met, and His residues, (iv) reactions of bound platinum with thiourea, and (v) gel filtration chromatography. In contrast to previous reports, it is shown that the major sulfur-containing binding site involves Met and not **Cys-34**, and also a N ligand, in the form of an S,N macrochelate. Additional monofunctional adducts involving other Met residues and **Cys-34** are also observed. During the later stages of reactions of cisplatin with albumin, release of NH3 occurs due to the strong trans influence of Met sulfur, which weakens the Pt-NH3 bonds, and protein cross-linking is observed. The consequences of these findings for the biological activity of cisplatin-albumin complexes are discussed.

L3 ANSWER 7 OF 39 MEDLINE on STN

1998246218. PubMed ID: 9586806. Photodynamically generated bovine **serum albumin** radicals: evidence for damage transfer and oxidation at cysteine and tryptophan residues. Silvester J A; Timmins G S; Davies M J. (Department of Chemistry, University of York, UK.) Free radical biology & medicine, **(1998 Mar 15)** 24 (5) 754-66. Journal code: 8709159. ISSN: 0891-5849. Pub. country: United States. Language: English.

AB Porphyrin-sensitized photoxidation of bovine **serum albumin** (BSA) results in oxidation of the protein at (at least) two different, specific sites: the **Cys-34** residue giving rise to a thiyl radical (RS.); and one or both of the tryptophan residues (Trp-134 and Trp-214) resulting in the formation of tertiary carbon-centred radicals and disruption of the tryptophan ring system. In the case of porphyrins such as hematoporphyrin, which bind at specific sites on BSA, these species appear to arise via long-range transfer of damage within the protein structure, as the binding site is some distance from the ultimate site of radical formation. This transfer of damage is shown to depend on a number of factors including the conformation of the protein, the presence of blocking groups and pH. Alteration of the protein conformation results in radical formation at additional (or alternative) sites, as does blocking of the preferred loci of radical formation. The formation of these thiyl and tryptophan-derived radicals does not lead to significant aggregation or fragmentation of the protein, though it does result in a dramatic enhancement in the susceptibility of the oxidised protein to proteolytic degradation by a range of proteases. The generation of protein-derived radicals also results in an enhancement of photobleaching of the porphyrin, suggesting that protein radical generation is linked to porphyrin photooxidation.

L3 ANSWER 8 OF 39 MEDLINE on STN
1998141963. PubMed ID: 9473299. Protein hydroperoxides and carbonyl groups generated by porphyrin-induced photo-oxidation of bovine **serum albumin**. Silvester J A; Timmins G S; Davies M J. (Department of Chemistry, University of York, York, United Kingdom.) Archives of biochemistry and biophysics, **(1998 Feb 15)** 350 (2) 249-58. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB Porphyrin-sensitized photo-oxidation of bovine **serum albumin** results in oxidation at specific sites to produce protein radical species: at the **Cys-34** residue (to give a thiyl radical) and at one or both tryptophan residues (Trp-134 and Trp-214) to give tertiary carbon-centered radicals and cause disruption of the indole ring system. This study shows that these photo-oxidation processes also consume oxygen and give rise to hydrogen peroxide, protein hydroperoxides, and carbonyl functions. The yield of hydrogen peroxide, protein hydroperoxides, and carbonyl functions is shown to be dependent on illumination time, the nature of the sensitizer, and the concentration of oxygen; the yield of hydroperoxides can also be markedly diminished by the presence of a spin trap which reacts with the initial protein radicals. The mechanism of formation of the protein hydroperoxides is suggested to be primarily through type I processes (i.e., independent of singlet oxygen), while type II (singlet oxygen) mechanisms may play a significant role in protein carbonyl formation. Reaction of the protein hydroperoxide species with metal ion complexes is shown to produce further protein-derived radicals which are predominantly present on amino acid side chains.

L3 ANSWER 9 OF 39 MEDLINE on STN
97480748. PubMed ID: 9339393. pH-dependent regulation of leukocyte 5-lipoxygenase activity in inflammatory exudates by albumin. Benz M; Werz O; Jacob R; Steinhilber D. (Institute of Pharmaceutical Chemistry, University of Frankfurt, Germany.) Inflammation research : official journal of the European Histamine Research Society ... [et al.], **(1997 Sep)** 46 (9) 366-72. Journal code: 9508160. ISSN: 1023-3830. Pub. country: Switzerland. Language: English.

AB OBJECTIVE AND DESIGN: In order to study the regulation of cellular 5-lipoxygenase activity under inflammatory conditions, the effects of inflammatory exudates on rat leukocyte 5 lipoxygenase activity were investigated. MATERIALS: Peritoneal leukocytes and inflammatory exudates were collected from glycogen treated rats. TREATMENT: Glycogen (1 g/kg body weight, in a final volume of 3 ml PBS) was injected intraperitoneally into male Wistar rats. After 4 h, the inflammatory exudate was collected. METHODS: Rat peritoneal leukocytes were isolated and the cellular 5-lipoxygenase activity was determined by HPLC after cell stimulation with calcium ionophore A23187. RESULTS: Inflammatory exudates from glycogen treated animals strongly inhibited cellular 5-lipoxygenase activity of ionophore challenged leukocytes. Albumin was identified as the inhibitor in exudates. Inhibition of cellular 5-lipoxygenase activity by albumin was pH-dependent and was strongly increased by the alkaline pH (7.9-8.0) of the exudate. The albumin effect increased in the range of pH 7.4-8.2 where albumin undergoes a conformational change called neutral to base (N-B) transition. S-Carboxymethyl-albumin had a similar activity to that of albumin, which indicated that the free SH-group at **Cys-34** of albumin is not necessary for the effect. The albumin dimer showed a significantly higher inhibition than albumin and it suppressed cellular 5-lipoxygenase activity by 98%. Peptic and tryptic fragments of albumin which comprise domains I, II and II, III, respectively, were less active or inactive. Thus, an intact albumin molecule or the dimer are required for efficient inhibition of cellular 5-lipoxygenase activity. CONCLUSIONS: Our data suggest that during inflammation, albumin extravasation and changes in pH-value are involved in the regulation of the inflammatory reaction by suppression of leukotriene release.

L3 ANSWER 10 OF 39 MEDLINE on STN
97452481. PubMed ID: 9308895. Effect of nitric oxide on the ligand-binding activity of albumin. Kashiba-Iwatsuki M; Miyamoto M; Inoue M. (Department of Biochemistry, Osaka City University Medical School, Osaka, Japan.) Archives of biochemistry and biophysics, **(1997 Sep 15)** 345 (2) 237-42. Journal code: 0372430. ISSN: 0003-9861. Pub. country: United States. Language: English.

AB The redox state of the **Cys-34** on albumin plays an important role in ligand binding of this plasma protein. We previously reported that mixed-disulfide formation of albumin with low molecular weight thiols, such as cysteine and glutathione, increased the affinity of this protein for phenolsulfophthalein (PSP) and Cu(II). Although nitric oxide (NO) and its metabolites easily react with various thiols, including that of albumin, and form S-nitrosothiol derivatives, the effect of such

modification on the ligand-binding activity of this plasma protein remains to be elucidated. Kinetic analysis revealed that S-nitrosylation of **Cys-34** on bovine **serum albumin** (BSA) decreased its binding activity for PSP. NO also decreased the ligand-binding activity of fresh plasma samples from rat and human. S-nitrosylation also decreased the binding activity of BSA for Cu(II). These results indicate that reversible modification of the **Cys-34** by NO and oxidative stress might play regulatory roles in the binding and transport of organic anions and heavy metals in the circulation.

L3 ANSWER 11 OF 39 MEDLINE on STN

97356266. PubMed ID: 9212708. Characterization of the glycation of albumin in freeze-dried and frozen human serum. Bunk D M. (National Institute of Standards and Technology, Galthersburg, Maryland 20899, USA.) Analytical chemistry, **(1997 Jul 1)** 69 (13) 2457-63. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB Human **serum albumin** (HSA) in fresh frozen and freeze-dried serum reference materials was examined by mass spectrometry and a variety of affinity chromatography techniques. The relative molecular mass distribution of HSA in fresh frozen serum was found to be identical to that of an HSA standard. However, the HSA in the freeze-dried reference serum exhibited a relative molecular mass distribution that was shifted to higher mass, broader, and substantially more heterogeneous than that of HSA in fresh frozen serum. A proteolytic cyanogen bromide digestion of the HSA from freeze-dried serum contained adducts approximately 162 u higher in mass than digest fragments 124-298 and 447-548, suggesting glycation. The presence of glycation on fragments 124-298 and 447-548 correlates with the known sites of HSA glycation. Glycation was further confirmed by the mass spectral analysis of the retained and unretained fractions from glycoaffinity chromatography of HSA from freeze-dried serum. The relative molecular weight of the HSA in the retained fraction indicated the presence of a doubly glycated species. The chemical heterogeneity of **Cys-34**, the site of the only free thiol in HSA, was examined and found not to be a substantial source of molecular mass heterogeneity for HSA from either fresh frozen of freeze-dried serum.

L3 ANSWER 12 OF 39 MEDLINE on STN

97321115. PubMed ID: 9177846. Structural determination of the conjugate of human **serum albumin** with a mitomycin C derivative, KW-2149, by matrix-assisted laser desorption/ionization mass spectrometry. Yasuzawa T; Tomer K B. (Laboratory of Structural Biology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709, USA.) Bioconjugate chemistry, **(1997 May-Jun)** 8 (3) 391-9. Journal code: 9010319. ISSN: 1043-1802. Pub. country: United States. Language: English.

AB A new mitomycin C derivative, KW-2149, is known to form a covalent conjugate with human **serum albumin** (HSA). This conjugate exhibits 1/20 of the anticellular activity of unconjugated KW-2149. Structural studies of this conjugate were carried out using a combination of enzymatic digestion, high-performance liquid chromatography (HPLC), and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. The tryptic peptide T5 (residues 21-41) was the only peptide found to be modified by KW-2149 moieties, the [(gamma-L-glutamylamino)ethyl]thio group or the (2-aminoethyl)thio group, through a disulfide bond. Although the latter peptide lost its mitomycin C moiety in the course of tryptic digestion, these data strongly suggest that KW-2149 was bound to **Cys-34**, the only free cysteine on HSA.

L3 ANSWER 13 OF 39 MEDLINE on STN

97273972. PubMed ID: 9128146. Probing the **cysteine 34** residue in human **serum albumin** using fluorescence techniques. Narazaki R; Maruyama T; Otagiri M. (Faculty of Pharmaceutical Sciences, Kumamoto University, Japan.) Biochimica et biophysica acta, **(1997 Apr 4)** 1338 (2) 275-81. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB The microenvironment surrounding **Cys-34** in human **serum albumin** (HSA) has been studied using acrylodan, a cysteine-specific fluorescence probe. Fluorescence emission maximum (Em(max)) of acrylodan covalently attached to **Cys-34** was observed at 476 nm, which was nearly the same as for acetonitrile. The Em(max) exhibited a shift toward longer wavelength with oleate binding. The acrylodan fluorescence of HSA exhibited heterogeneous decay kinetics, which adequately fit a double-exponential decay model. When three or more oleate molecules were bound to one albumin molecule, the fractional intensity was shifted in favor of the shorter lived component. These results suggest that oleate binding induces a conformational or dynamic change which is localized in the **Cys-34** region. Unfolding studies with guanidine-HCl indicate that **Cys-34** appears to be located on the surface of HSA molecule and that it is protected by adjacent amino acid residues. Solvent accessibility of

acrylodan with HSA in the absence and presence of oleate was determined from acrylamide quenching, and suggests that oleate binding enhances the solvent exposure of the acrylodan fluorophore. In order to determine the nature of the electrostatic potential near **Cys-34**, the quenching rate constants for anionic (iodide) and cationic (thallium) quenchers were determined as a function of ionic strength of solvent. The ionic strength dependence of quenching indicated that there was an electrostatic attractive force between the fluorophore and both ionic quenchers. These results are consistent with a model in which amphoteric charges which arise from charged amino acid residues were surrounding **Cys-34**. Interestingly, oleate binding resulted in changes in the spatial relationships between acrylodan and these charged residues. Thus, the experiments described herein provide the information concerning an oleate-induced alteration in the nature of the local environment surrounding **Cys-34** and suggests that long chain fatty acid binding provides a method for regulating the radical-trapping antioxidant activity of **Cys-34** in HSA in vivo.

L3 ANSWER 14 OF 39 MEDLINE on STN

96390393. PubMed ID: 8797379. Accessibility of the fluorescent reporter group in native, silica-adsorbed, and covalently attached acrylodan-labeled serum albumins. Ingersoll C M; Jordan J D; Bright F V. (Department of Chemistry, State University of New York at Buffalo 14260-3000, USA.) Analytical chemistry, **(1996 Sep 15)** 68 (18) 3194-8. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB Fluorescence quenching techniques are used to investigate the accessibility of a model biorecognition element-reporter group system when in buffer, surface-adsorbed, and covalently attached to a silica surface. The site-selective fluorescent reporter group, 6-acryloyl(dimethylamino)naphthalene (acrylodan, Ac), is attached covalently (at **cysteine-34**) to bovine and human **serum albumin** (BSA and HSA, respectively) and serves as a surrogate recognition element-reporter group system. Molecular oxygen is used to quench the Ac fluorescence and the accessibility, in the form of bimolecular rate constants (kq), in each model system is quantified. Although one might expect these systems to exhibit similar behavior, differences in quenching characteristics are observed, such as wavelength dependency of the Stern-Volmer quenching constant (KSV) for the native proteins in buffer. BSA-Ac exhibits wavelength dependent KSV values as well as a blue-shifted emission spectrum on O2 addition. Physisorption of BSA-Ac onto a fused-silica optical fiber lowers the accessibility of Ac to O2, whereas covalent attachment of BSA-Ac to APTES/glutaraldehyde-modified silica enhances the accessibility of the Ac reporter group to O2.

L3 ANSWER 15 OF 39 MEDLINE on STN

96299075. PubMed ID: 8660549. S-nitrosation of **serum albumin**: spectrophotometric determination of its nitrosation by simple S-nitrosothiols. Zhang H; Means G E. (Department of Biochemistry, Ohio State University, 484 West 12th Avenue, Columbus, Ohio, 43210, USA.) Analytical biochemistry, **(1996 May 15)** 237 (1) 141-4. Journal code: 0370535. ISSN: 0003-2697. Pub. country: United States. Language: English.

AB The transfer of nitroso groups from S-nitroso-L-cysteine (1) and six other simple S-nitrosothiols to **Cys 34** of bovine **serum albumin** (2) has been followed using Ellman's reagent, 5,5'-dithio-bis (2-nitrobenzoate) (3), to detect the resulting thiols. The described method utilizes the low reactivity of (3) with (2) and the high extinction coefficient of 2-nitro-5-thiobenzoate produced upon its reaction with thiols to follow the transfer of nitroso moieties at low concentrations where other procedures are not feasible. A second-order rate constant of 6400 M-1 s-1 obtained for the reaction of (2) with S-nitrosomercaptoethylamine is approximately 10 times faster than that for its reaction with (1), approximately 40 times faster than that for its reaction with S-nitrosoglutathione, and consistent with **Cys 34** being located in a narrow crevice in close proximity to an anionic charge.

L3 ANSWER 16 OF 39 MEDLINE on STN

96229016. PubMed ID: 8686877. Dynamics of acrylodan-labeled bovine and human **serum albumin** entrapped in a sol-gel-derived biogel. Jordan J D; Dunbar R A; Bright F V. (Department of Chemistry, Natural Sciences and Mathematics Complex, State University of New York at Buffalo 14260-3000, USA.) Analytical chemistry, **(1995 Jul 15)** 67 (14) 2436-43. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB We investigate acrylodan-labeled bovine and human **serum albumin** (BSA-Ac and HSA-Ac) entrapped within a tetramethylorthosilane-derived biogel composite. The effects of biogel aging and drying were studied by following the acrylodan steady-state and time-resolved emission, the decay of anisotropy, and the dipolar relaxation kinetics as a function of

ambient storage time. The results indicate that there is a substantial amount of nanosecond and subnanosecond dipolar relaxation within the local environment surrounding **cysteine-34** in both proteins, even when they are fully encapsulated in a dry biogel. Time-resolved anisotropy experiments show that the acrylodan residue and the protein are able to undergo nanosecond motion within the biogel. The semiangle through which the acrylodan can process is the same for a freshly formed biogel and the native protein in buffer. However, once the biogel begins to dry, the semiangle increases (approximately 20 degrees and 10 degrees for BSA-Ac and HSA-Ac, respectively). This suggests that the "pocket" hosting the acrylodan reporter group opens as the biogel dries.

L3 ANSWER 17 OF 39 MEDLINE on STN

96062545. PubMed ID: 7586558. Calcium ion binding to clinically relevant chemical modifications of human **serum albumin**. Vorum H; Fisker K; Otagiri M; Pedersen A O; Kragh-Hansen U. (Department of Medical Biochemistry, University of Aarhus, Denmark.) Clinical chemistry, **(1995 Nov)** 41 (11) 1654-61. Journal code: 9421549. ISSN: 0009-9147. Pub. country: United States. Language: English.

AB Calcium binding to glycated, penicilloylated, acetylated, and normal defatted human **serum albumin** as well as to mercapt- and nonmercaptalbumin was studied by equilibrium dialysis of radioactive Ca2+. Binding was quantified by five Scatchard constants [ni = 1, (i = 1-4) and n5 = 10]. Glycation resulted in increased k1- and k2-values and unchanged k3-k5-values, whereas penicilloylation increased all five association constants. The increments were greater the more pronounced the modification, and the enhancements caused by penicilloylation were, for the same degree of modification, greater than those produced by glycation. In contrast, acetylation by acetylsalicylate did not affect calcium binding. Likewise, binding to mercapt- and nonmercaptalbumin was the same, a finding showing that the thiol group of **cysteine 34** is not important for calcium binding. D-Glucose and penicillin G are known to react with lysine residues of albumin, and the enhancement of binding resulting from glycation or penicilloylation is probably brought about by unspecific electrostatic effects, possibly supplemented by conformational changes of the protein molecule. The relative importance of the three domains of human **serum albumin** for calcium binding is discussed.

L3 ANSWER 18 OF 39 MEDLINE on STN

95168613. PubMed ID: 7864387. Dynamics surrounding **Cys-34** in native, chemically denatured, and silica-adsorbed bovine **serum albumin**. Wang R; Sun S; Bekos E J; Bright F V. (Department of Chemistry, Natural Sciences and Mathematics Complex, State University of New York at Buffalo 14260-3000.) Analytical chemistry, **(1995 Jan 1)** 67 (1) 149-59. Journal code: 0370536. ISSN: 0003-2700. Pub. country: United States. Language: English.

AB We report the steady-state and time-resolved fluorescence of 6-acryloyl(dimethylamino)naphthalene (acrylodan) covalently attached to **Cys-34** in bovine **serum albumin** (BSA). For this conceptually simple system, complicated fluorescence intensity and anisotropy decay kinetics are observed. The steady-state and time-resolved results demonstrate the presence of an excited-state reaction for the BSA-acrylodan system. Additional analysis shows that dipolar relaxation of the environment surrounding acrylodan within BSA is responsible for most of the observed time-dependent evolution of the emission spectrum. The effects of temperature, chemical denaturation, and protein adsorption to a bare silica substrate are also investigated. These results demonstrate the complexity of the changes within a protein/biorecognition element that affect the signal from a single fluorescent reporter group.

L3 ANSWER 19 OF 39 MEDLINE on STN

95017307. PubMed ID: 7931850. Conformational changes induced in bovine **serum albumin** by the photodynamic action of haematoporphyrin. Timmins G S; Davies M J. (Department of Chemistry, University of York, Heslington, UK.) Journal of photochemistry and photobiology. B, Biology, **(1994 Jul)** 24 (2) 117-22. Journal code: 8804966. ISSN: 1011-1344. Pub. country: Switzerland. Language: English.

AB The photodynamic action of haematoporphyrin upon bovine **serum albumin**, spin-labelled at the **cysteine-34** residue, has been shown to: (i) increase its susceptibility to proteolysis by chymotrypsin and trypsin, and (ii) increase its susceptibility to denaturation by urea. This is thought to be the result of conformational changes caused by the formation of protein radicals, although contributions from subsequent radical reactions of, for example, amino acids, may also take place. Such species have previously been shown, by EPR spin-trapping, to be formed in this system. Increased proteolytic susceptibility of non spin-labelled protein is also observed upon photolysis with haematoporphyrin, indicating that the changes observed in the spin-labelled protein also occur in the native form, and are not artefactual in nature. The significance of these

photochemically-induced conformational changes within proteins in the photodynamic therapy of tumours, and other protein-radical systems is discussed.

L3 ANSWER 20 OF 39 MEDLINE on STN

94207012. PubMed ID: 8155715. The kinetic studies on the intramolecular SH, S-S exchange reaction of bovine mercaptalbumin. Kuwata K; Era S; Sogami M. (Department of Physiology, School of Medicine, Gifu University, Japan.) Biochimica et biophysica acta, **(1994 Apr 13)** 1205 (2) 317-24. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Bovine mercaptalbumin (BMA) has 17 disulfide bonds and one SH group at **Cys-34** which catalyzes the intramolecular SH, S-S exchange reaction (N-A isomerization, molecular aging) in the alkaline region at low ionic strength, resulting in the formation of the aged form (A-form). The aging reaction was completely reversible and strongly affected by environmental factors, such as pH, temperature, ionic strength, Ca2+, nonbranched short-chain fatty acids, etc. Disulfide configuration (or pairing of disulfide bonds) was affected by the environmental factors. Obtained results might support the concept of Klotz (1966) that protein conformation (or three-dimensional structure) is dependent upon (i) the primary structure and (ii) constituents of the solvent.

L3 ANSWER 21 OF 39 MEDLINE on STN

93307207. PubMed ID: 8319609. Metabolism of the food-borne carcinogens 2-amino-3-methylimidazo-[4,5-f]quinoline and 2-amino-3,8-dimethylimidazo[4,5-f]-quinoxaline in the rat as a model for human biomonitoring. Turesky R J; Stillwell W G; Skipper P L; Tannenbaum S R. (Nestec Ltd., Nestle Research Center, Lausanne, Switzerland.) Environmental health perspectives, **(1993 Mar)** 99 123-8. Journal code: 0330411. ISSN: 0091-6765. Pub. country: United States. Language: English.

AB Metabolism of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and 2-amino-3,8-dimethylimidazol[4,5-f]quinoxaline (MeIQx) and their binding to blood proteins were examined in the rat to develop methods of human biomonitoring. Hemoglobin and **serum albumin** were among many blood proteins modified. Approximately 0.01% of the dose for both compounds was bound to these proteins, and induction of cytochrome P-450 with polychlorobiphenyls resulted in decreased levels of adduction. Hemoglobin sulfinic acid amide adducts could not be detected for either amine, however, as much as 10% of the IQ bound to albumin was characterized as an N2-**cysteine**(**34**)sulfinyl-IQ linkage. Human dosimetry of these carcinogens through such adducts may prove difficult due to the low levels of protein binding. Major routes of detoxification of both contaminants included cytochrome P-450-mediated ring hydroxylation at the C-5 position followed by conjugation to glucuronic or sulfuric acid. Direct conjugation to the exocyclic amine group through N-glucoronidation and sulfamate formation were other important routes of inactivation, but N-acetylation was a minor pathway. The N-glucoronide conjugate of the mutagenic metabolite N-hydroxy-MeIQx was also detected in urine. Rats given MeIQx at 10 micrograms/kg excreted 20% of the dose in urine within 24 hr and the remainder was recovered in feces. The N2-glucuronide was the major metabolite found in urine and accounted for 4% of the total dose. The other metabolites cited above also were excreted in urine at amounts ranging from 0.5 to 3% of the dose, whereas 0.5 to 2% was detected as unmetabolized MeIQx.(ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 22 OF 39 MEDLINE on STN

92366524. PubMed ID: 1502182. Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of **serum albumin**. Stamler J S; Jaraki O; Osborne J; Simon D I; Keaney J; Vita J; Singel D; Valeri C R; Loscalzo J. (Department of Medicine, Harvard University, Cambridge, MA 02138.) Proceedings of the National Academy of Sciences of the United States of America, **(1992 Aug 15)** 89 (16) 7674-7. Journal code: 7505876. ISSN: 0027-8424. Pub. country: United States. Language: English.

AB We have recently shown that nitric oxide or authentic endothelium-derived relaxing factor generated in a biologic system reacts in the presence of specific protein thiols to form S-nitrosoprotein derivatives that have endothelium-derived relaxing factor-like properties. The single free cysteine of **serum albumin, Cys-34**, is particularly reactive toward nitrogen oxides (most likely nitrosonium ion) under physiologic conditions, primarily because of its anomalously low pK; given its abundance in plasma, where it accounts for approximately 0.5 mM thiol, we hypothesized that this plasma protein serves as a reservoir for nitric oxide produced by the endothelial cell. To test this hypothesis, we developed a methodology, which involves UV photolytic cleavage of the S--NO bond before reaction with ozone for chemiluminescence detection, with which to measure free nitric oxide, S-nitrosothiols, and S-nitrosoproteins in biologic systems. We found that human plasma contains approximately 7 microM S-nitrosothiols, of which 96% are

S-nitrosoproteins, 82% of which is accounted for by S-nitroso-**serum albumin**. By contrast, plasma levels of free nitric oxide are only in the 3-nM range. In rabbits, plasma S-nitrosothiols are present at approximately 1 microM; 60 min after administration of NG-monomethyl-L-arginine at 50 mg/ml, a selective and potent inhibitor of nitric oxide synthetases, S-nitrosothiols decreased by approximately 40% (greater than 95% of which were accounted for by S-nitrosoproteins, and approximately 80% of which was S-nitroso-**serum albumin**); this decrease was accompanied by a concomitant increase in mean arterial blood pressure of 22%. These data suggest that naturally produced nitric oxide circulates in plasma primarily complexed in S-nitrosothiol species, principal among which is S-nitroso-**serum albumin**. This abundant, relatively long-lived adduct likely serves as a reservoir with which plasma levels of highly reactive, short-lived free nitric oxide can be regulated for the maintenance of vascular tone.

L3 ANSWER 23 OF 39 MEDLINE on STN

92240092. PubMed ID: 1368072. Immobilized metal ion affinity chromatography of serum albumins. Andersson L; Sulkowski E; Porath J. (Institute of Biochemistry and Biochemical Separation Center, Uppsala University, Sweden.) Bioseparation, **(1991)** 2 (1) 15-22. Journal code: 9011423. ISSN: 0923-179X. Pub. country: Netherlands. Language: English.

AB The interaction of several serum albumins with chelated (iminodiacetate, IDA) and immobilized (agarose-IDA) metal ions, Co2+, Ni2+, Cu2+ and Zn2+, was studied. There was no retention of human, bovine, porcine, murine and avian albumins on IDA-Zn(II) and IDA-Co(II) columns. However, all albumins studied, i.e., those of: man, cow, pig, dog, rabbit, rat, mouse, chicken and pigeon were retained on IDA-Cu(II) columns, and all except dog albumin were retained also on IDA-Ni(II). The recognition of albumins by chelated and immobilized transition metals seems to be related to an affinity for the imidazole side chains. It is postulated that one to three imidazoles is involved in this interaction, under the employed experimental conditions (pH 7.0; 1 M sodium chloride). There is no evidence for any significant contribution of tryptophan or cysteine (**Cys 34**) residues to the chromatographic event. The retention of defatted albumin and albumin oligomers (human), on IDA-Cu(II) columns was not significantly different from that of non-defatted albumin or albumin monomer, respectively.

L3 ANSWER 24 OF 39 MEDLINE on STN

92191338. PubMed ID: 1799947. Multiple and irreversible binding of cis-diamminedichloroplatinum(II) to human **serum albumin** and its effect on warfarin binding. Yotsuyanagi T; Ohta N; Futo T; Ito S; Chen D N; Ikeda K. (Faculty of Pharmaceutical Sciences, Nagoya City University, Japan.) Chemical & pharmaceutical bulletin, **(1991 Nov)** 39 (11) 3003-6. Journal code: 0377775. ISSN: 0009-2363. Pub. country: Japan. Language: English.

AB Irreversible bindings of cis-diamminedichloroplatinum(II) (cis-DDP) to human **serum albumin** (HSA) were investigated in a pH 7.4 buffer containing 0.1 M NaCl at various molar ratios (cis-DDP/HSA) up to 60 over a 14 d period (37 degrees C). The metal binding seemed to reach a plateau when incubated at less than 10 times excess of cis-DDP. As the molar ratio increased, the reaction rate was relatively fast within the first day, followed by a moderate increase in the metal binding. When incubated at 60 times excess of cis-DDP, the metal bound as much as 20 mol per mol of HSA in 14 d. Fluorescence quenching of the metal-bound protein suggested that the tryptophan residue was gradually exposed to a hydrophilic environment as the metal binding increased. Furthermore, cis-DDP cleaved disulfide bonds at the ratio of 1 mol of disulfide bond per 5.3 mol of the metal binding. It was therefore suggested that the metal binding also occurred at several sites other than the disulfide bond. Warfarin binding to the metal-bound protein, examined by fluorescence changes, also decreased with increasing metal binding or cleavage of the disulfide bonds. Thus, cis-DDP bound to multiple sites in addition to the lone sulfhydryl group (**Cys-34**), suggesting that massive conformational changes of the protein took place.

L3 ANSWER 25 OF 39 MEDLINE on STN

92104776. PubMed ID: 1761372. Circular dichroic and 1H-NMR studies on the aged form of bovine plasma albumin. Era S; Kuwata K; Sogami M; Kato K; Watari H. (Department of Physiology, School of Medicine, Gifu University, Japan.) International journal of peptide and protein research, **(1991 Sep)** 38 (3) 260-6. Journal code: 0330420. ISSN: 0367-8377. Pub. country: Denmark. Language: English.

AB Bovine plasma albumin (BPA) has 17 disulfide bonds and approximately one SH group at **Cys-34** which catalyzes the intramolecular SH, S-S exchange reaction in the alkaline region at low ionic strength, resulting in the formation of the aged form (A-form). 1) Fractions of alpha-helix (f alpha) and beta-form (f beta) of iodoacetamide-blocked non-aged BPA (IA-BPA) at pH 6.5 (the N-form) and 9.0 (the B-form) in the absence of added salt were

0.70, 0.12 and 0.62, 0.18, respectively (Era et al. (1990]. However, there were no changes in f alpha and f beta of the iodoacetamide-blocked A-form (IA-A-form) over the pH range from 5.5 to 9.1 in the absence of added salt or in 0.10 M KCl (f alpha approximately 0.60, f beta approximately 0.20), indicating that the secondary structure of the IA-A-form might be similar to that of non-aged IA-BPA at pH 9.0 (B-form) in the absence of added salt, that is, the frozen B-form, stabilized covalently by the repairing of disulfide bonds. 2) The rigidity of the A- and IA-A-forms, as monitored by cross-relaxation times between irradiated and observed protein protons, was similar to or slightly higher than that of non-aged IA-BPA or BMA.(ABSTRACT TRUNCATED AT 250 WORDS)

L3 ANSWER 26 OF 39 MEDLINE on STN

92003197. PubMed ID: 1912331. Carbamoylation of peptides and proteins in vitro by S-(N-methylcarbamoyl)glutathione and S-(N-methylcarbamoyl)cysteine, two electrophilic S-linked conjugates of methyl isocyanate. Pearson P G; Slatter J G; Rashed M S; Han D H; Baillie T A. (Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle 98195.) Chemical research in toxicology, **(1991 Jul-Aug)** 4 (4) 436-44. Journal code: 8807448. ISSN: 0893-228X. Pub. country: United States. Language: English.

AB The reactivity toward peptides and proteins of S-(N-methylcarbamoyl)glutathione (SMG), the glutathione conjugate of methyl isocyanate, and the corresponding cysteine adduct, S-(N-methylcarbamoyl)cysteine (SMC), was investigated with the aid of in vitro model systems. Incubation of SMC or a trideuteriomethyl analogue of SMC with either the reduced or oxidized forms of oxytocin afforded similar mixtures of mono-, bis- and tris-N-methylcarbamoylated peptides. Structure elucidation of the mono and bis adducts by fast atom bombardment tandem mass spectrometry indicated that carbamoylation of oxytocin occurred preferentially at Cys-6 and that Cys-1 and/or Tyr-2 were secondary sites of modification. Upon incubation of S-[N-([14C]methyl)carbamoyl]glutathione (14C-SMG) with native bovine **serum albumin** (BSA), radioactivity became bound covalently to the protein in a time- and concentration-dependent fashion. "Blocking" of the lone **Cys-34** thiol group of BSA in the form of a disulfide prior to exposure of the protein to 14C-SMG failed to decrease significantly the extent or time course of this covalent binding. It is concluded that carbamate thioester conjugates of MIC are reactive, carbamoylating entities which can donate the elements of MIC to nucleophilic functionalities on peptides and proteins. Free thiols appear to be preferred sites for such carbamoylation processes, a phenomenon that may have important toxicological consequences in the pathology of tissue lesions induced by MIC and related isocyanates.

L3 ANSWER 27 OF 39 MEDLINE on STN

91298263. PubMed ID: 1676877. Application of a novel 1:1 gold(I)-chromophoric thiolate complex as a spectrophotometric probe for the thiol-exchange reactions of anti-arthritic gold drugs in biological fluids. Grootveld M; Claxson A W; Furst A; Blake D R. (Inflammation Group, London Hospial Medical College.) Agents and actions. Supplements, **(1991)** 32 77-81. Journal code: 7801014. ISSN: 0379-0363. Pub. country: Switzerland. Language: English.

AB An oligomeric 1:1 gold(I) complex of the chromophoric thiol 5-mercapto (2-nitrobenzoate) has been synthesized and applied as a spectrophotometric probe for the thiol-exchange reactions of structurally-analogous 1:1 gold(I)-thiolate drugs. For low-molecular-mass thiols, results were consistent with the initial formation of a monomeric mixed-ligand bis-thiolato gold(I) complex followed by further ligand substitution by excess thiol to produce 5-mercapto (2-nitrobenzoate) and a monomeric bis-thiolato gold(I) complex. For human **serum albumin**, however, the spectrophotometric changes were only consistent with the binding of gold(I) to its single cysteine residue (**Cys-34**) with the retention of the 5-mercapto (2-nitrobenzoate) ligand on gold(I).

L3 ANSWER 28 OF 39 MEDLINE on STN

91214264. PubMed ID: 2090031. Interaction of methylmercury compounds with albumin. Yasutake A; Hirayama K; Inoue M. (Biochemistry Section, National Institute for Minamata Disease, Kumamoto, Japan.) Archives of toxicology, **(1990)** 64 (8) 639-43. Journal code: 0417615. ISSN: 0340-5761. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The nature of interaction between bovine **serum albumin** (BSA) and methylmercurial compounds has been investigated by ultrafiltration analysis. Four types of BSA samples, mercaptalbumin, its mixed disulfides with glutathione (GSH) and L-cysteine (CySH), and S-carbamidomethylated derivative, were used for binding assays with methylmercury (MM) chloride (MMC) and three kinds of MM mercaptides of low molecular weight thiols, GSH (GS-MM), CySH (CyS-MM) and cysteinylglycine (CG-MM). Among various ligands tested, MMC showed the highest affinity for all BSA species, and

the BSA-bound fraction of the ligand did not change with ligand/protein ratio. MMC strongly and stoichiometrically bound to mercaptalbumin even at a molar ratio of 1:1. In contrast, the albumin bound fractions of three other MM ligands increased with concomitant decrease in ligand/protein ratio and with time except for the alkylated albumin, the highest binding being shown by mercaptalbumin. Binding of S-2-nitrophenyl-glutathione, a GSH analog with a hydrophobic S-substituent, to albumin species occurred similarly to that of GS-MM. However, GSH and oxidized glutathione (GSSG) interacted differently with albumin; mercaptalbumin showed the lowest affinity for GSH, and GSSG scarcely interacted with all BSA species. These results suggest that the sulfhydryl group at **Cys-34** is not the only site of BSA that interacts with MM compounds and that albumin interacts preferentially with the hydrophobic domains of a mercurial ligand rather than its hydrophilic peptide moiety.

L3 ANSWER 29 OF 39 MEDLINE on STN

90216119. PubMed ID: 2323880. Structural transition of bovine plasma albumin in the alkaline region--the N-B transition. Era S; Itoh K B; Sogami M; Kuwata K; Iwama T; Yamada H; Watari H. (Department of Physiology, School of Medicine, Gifu University, Japan.) International journal of peptide and protein research, **(1990 Jan)** 35 (1) 1-11. Journal code: 0330420. ISSN: 0367-8377. Pub. country: Denmark. Language: English.

AB Bovine plasma albumin (BPA) has approximately one SH group (**Cys-34**) which catalyzes the intramolecular SH, S-S exchange reaction in the alkaline region at low ionic strength, resulting in the formation of the aged form. So, the N-B transition at ionic strength above 0.20 and below 0.10 was studied using BPA and iodoacetamide-blocked BPA (IA-BPA), respectively. (1) pH profiles of [theta]262 and [theta]268 of BPA in 0.20 M KCl showed the characteristic changes in the pH region 7.0-9.0, corresponding to the N-B transition. On going from pH 7.0 to 9.0 in 0.10 M KCl or NaCl, IA-BPA did not show significant changes in rotational relaxation times of tryptophyl fluorophors, CD-resolved secondary structures, spin-echo 1H-n.m.r. spectra and cross-relaxation times (TIS) between irradiated and observed protein protons, which might reflect the rigidity of the domains and/or subdomains. On the other hand, rotational relaxation times of 1-anilino-8-naphthalenesulfonate-IA-BPA complex (IA-BPA-ANS0.9, molar ratio of ANS to IA-BPA = 0.9/1) showed significant decreases from 131 to 114 ns on going from the N- to the B-forms in 0.10 M KCl. The above results and reported experimental evidence might indicate that on going from the N- to the B-forms in 0.10 M KCl or NaCl, the mutual movement of subdomains, connected with a flexible hinge region (Brown & Shockley (1982)) might increase without loss in the helicity and the rigidity of subdomains. (2) The N-B transition of IA-BPA in the absence of salt was quite different from those in 0.10 M KCl or NaCl. Decreases in the helicity and the intramolecular rigidity, as monitored by TIS-measurements, were observed on going from the N- to the B-forms.

L3 ANSWER 30 OF 39 MEDLINE on STN

88244667. PubMed ID: 2454291. Selectivity and stereospecificity of the reactions of dichlorodiammineplatinum(II) with three purified plasma proteins. Pizzo S V; Swaim M W; Roche P A; Gonias S L. (Department of Pathology, Duke University, Durham, North Carolina.) Journal of inorganic biochemistry, **(1988 May)** 33 (1) 67-76. Journal code: 7905788. ISSN: 0162-0134. Pub. country: United States. Language: English.

AB The reactions of cis- and trans-dichlorodiammineplatinum(II) (cis- and trans-DDP) with albumin and two plasma proteinase inhibitors were compared. Reaction with alpha 2-macroglobulin (alpha 2M) resulted in subunit crosslinking and loss of proteinase binding activity. The reaction also modified a receptor recognition site present on each alpha 2M subunit. While more trans-DDP was incorporated into alpha 2M than cis-DDP, cis-DDP was more effective at blocking receptor recognition, alpha 1-proteinase inhibitor was also inactivated by reaction with either cis- or trans-DDP. These reactions resulted in binding of platinum to methionine-358 at the reactive center of this inhibitor. Trans-DDP, however, was less selective and also bound to the single cysteine residue (Cys-232) of alpha 1PI. Reaction of albumin with cis-DDP resulted in incorporation of about 1 mol platinum per mol protein, and this platinum modified the single cysteine (**Cys-34**) in the molecule. Albumin incorporated twice as much trans-DDP, but the binding did not involve **cysteine-34**. In general, reactions of cis-DDP with proteins appear to be more selective than those observed for modification with the trans isomer.

L3 ANSWER 31 OF 39 MEDLINE on STN

88010021. PubMed ID: 3655788. Thiol competition for Et3PAuS-albumin: a nonenzymatic mechanism for Et3PO formation. Coffer M T; Shaw C F 3rd; Hormann A L; Mirabelli C K; Crooke S T. (Department of Chemistry,

University of Wisconsin, Milwaukee.) Journal of inorganic biochemistry, **(1987 Jul)** 30 (3) 177-87. Journal code: 7905788. ISSN: 0162-0134. Pub. country: United States. Language: English.

AB Thiols (RSH = 2,3,4,6-tetra-O-acetyl-beta-1-D-thioglucose, beta-1-D-thioglucose, and glutathione) can displace either the albumin or the triethylphosphine from the protein-gold complex, AlbSAuPEt3. The albumin is displaced in preference to triethylphosphine, but irreversible oxidation of the latter eventually shifts the equilibria toward Et3PO and AlbSAuSR. Albumin disulfide bonds are the probable oxidants. Neither O2 nor oxidized glutathione substantially enhanced the rate or extent of Et3PO formation. The labilization of the phosphine in AlbSAuPEt3 is attributed to a strong trans effect of the albumin thiolate, **Cys-34**. The 31P NMR chemical shifts of various thiolato(triethylphosphine)gold(I) complexes are correlated directly with the affinity of the thiols for gold and inversely with their pKSH values. Deacetylated auranofin (1-thio-beta-D-glucopyranosato-S) (triethylphosphine)gold(I) reacts with the mercaptalbumin and oxidized mercaptalbumin (putatively AlbSOH) forms of bovine **serum albumin** to form AlbSAuPEt3 with displacement of the thioglucose ligand.

L3 ANSWER 32 OF 39 MEDLINE on STN

87270873. PubMed ID: 3111487. Effect of protein concentration on the binding of gold(I) to human **serum albumin**. Pedersen S M. Biochemical pharmacology, **(1987 Aug 15)** 36 (16) 2661-6. Journal code: 0101032. ISSN: 0006-2952. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The binding of aurothiosulphate, gold(I), by human **serum albumin** has been studied by equilibrium dialysis at four different albumin concentrations, 37 degrees, pH 7.2-7.4 and ionic strength 0.15 M. The results show that the interaction of aurothiosulphate with albumin depends on albumin concentration. This observation is linked with the previous observation that the usual independent site description cannot be used to represent the clinically important low concentration data. All the observed dependences are satisfactorily accounted for by assuming that gold(I) competes with a highly bound contaminant for the high affinity (**Cys(34)**-SH) site. This description is supported by the experimental observation that a fraction of this site is originally blocked both in vivo and in in vitro. The present interpretation yields a high affinity binding constant 100 times larger than found previously and provides an explanation for the lack of correlation between dose and therapeutic and toxic effects in chrysotherapy.

L3 ANSWER 33 OF 39 MEDLINE on STN

87250634. PubMed ID: 3597434. Selective covalent labeling of cysteines in bovine **serum albumin** and in hepatoma tissue culture cell glucocorticoid receptors by dexamethasone 21-mesylate. Simons S S Jr. Journal of biological chemistry, **(1987 Jul 15)** 262 (20) 9669-75. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The specificity of protein labeling by an affinity label of glucocorticoid receptors, dexamethasone 21-mesylate (Dex-Mes), was investigated using bovine **serum albumin** (BSA) as a model. During the early stages of [3H]Dex-Mes labeling at pH 8.8, approximately 90% of the covalent bond formation occurred at the one non-oxidized cysteine (**Cys-34**) of BSA. The nonspecific labeling was equally distributed over the rest of the BSA molecule. [3H]Dex-Mes labeling of **Cys-34** was totally, and specifically inhibited by nearly stoichiometric amounts of the thiol-specific reagent methyl methanethiolsulfonate (MMTS). Thus both Dex-Mes and MMTS appear to react very selectively with thiols under our conditions. In reactions with hepatoma tissue culture (HTC) cell glucocorticoid receptors, MMTS was equally efficient in preventing [3H]dexamethasone binding to receptors and [3H]Dex-Mes labeling of the 98-kDa receptor protein. These results indicate that Dex-Mes labeling of the glucocorticoid receptor involves covalent reaction with at least one cysteine in the steroid binding site of the receptor. Small (approximately 1600-dalton) fragments of the [3H]Dex-Mes-labeled 98-kDa receptor were generated by limit proteolysis with trypsin, chymotrypsin, and Staphylococcus aureus V8 protease under denaturing conditions. Data from these fragments on 15% sodium dodecyl sulfate-polyacrylamide gels were consistent with all of the covalent [3H] Dex-Mes being located on one or a few cysteines in one approximately 15-residue stretch of the receptor. Further studies revealed no differences in the limit protease digestion patterns of activated and unactivated [3H]Dex-Mes-labeled receptors with trypsin, chymotrypsin, or V8 protease under denaturing conditions. These data suggest that activation does not cause any major covalent modifications of the amino acids immediately surrounding the affinity-labeled cysteine(s) of the steroid binding site.

L3 ANSWER 34 OF 39 MEDLINE on STN

85201880. PubMed ID: 3995650. Structural characterization of the major

covalent adduct formed in vitro between acetaminophen and bovine **serum albumin**. Hoffmann K J; Streeter A J; Axworthy D B; Baillie T A. Chemico-biological interactions, **(1985 Feb-Apr)** 53 (1-2) 155-72. Journal code: 0227276. ISSN: 0009-2797. Pub. country: Netherlands. Language: English.

AB The structure of the covalent adduct formed in vitro between [14C]-acetaminophen ([14C]APAP) and bovine **serum albumin** (BSA) has been investigated with the aid of new analytical methodology. The APAP-BSA adduct, isolated from mouse liver microsomal incubations to which the radiolabeled drug and BSA had been added, was cleaved using a combination of specific (cyanogen bromide) and non-specific (acid hydrolysis) procedures, following which the mixture of amino acids obtained was derivatized, in aqueous solution, with ethyl chloroformate. The resulting ethoxycarbonyl derivatives were recovered by extraction into ethylacetate, methylated and subjected to profile analysis using both reverse-phase and normal-phase HPLC techniques. In each HPLC step, one major radioactive amino acid adduct was detected and was identified by mass spectrometry as the derivative of 3-cystein-S-yl-4-hydroxyaniline. Based on this finding, and with a knowledge of the behavior under acidic hydrolysis conditions of the 3-cysteinyl conjugate of APAP, it could be concluded that the major APAP-BSA adduct is one in which the drug is bound, via a thioether linkage at the C-3 position, to a sulfhydryl group on the protein. Furthermore, it could be established that this -SH function almost certainly is that associated with the **cys-34** residue of BSA.

L3 ANSWER 35 OF 39 MEDLINE on STN

83231442. PubMed ID: 6860638. Resonance energy transfer between **cysteine-34**, tryptophan-214, and tyrosine-411 of human **serum albumin**. Hagag N; Birnbaum E R; Darnall D W. Biochemistry, **(1983 May 10)** 22 (10) 2420-7. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Reaction of p-nitrophenyl anthranilate with human **serum albumin** at pH 8.0 results in esterification of a single anthraniloyl moiety with the hydroxyl group of tyrosine-411. The absorption spectrum of the anthraniloyl group overlaps the fluorescence emission of the single tryptophan residue at position 214. This study complements that of the preceding paper [Suzukida, M., Le, H. P., Shahid, F., McPherson, R. A., Birnbaum, E.R., & Darnall, D. W. (1983) Biochemistry (preceding paper in this issue)] where an azomercurial group was introduced at **cysteine-34**. Anthraniloyl fluorescence was also quenched by the azomercurial absorption at **Cys-34**. Thus measurement of resonance energy transfer between these three sites allowed distances to be measured between **Cys-34** in domain I, Trp-214 in domain II, and Tyr-411 in domain III of human **serum albumin**. At pH 7.4 in 0.1 M phosphate the Trp-214 leads to Tyr-411, Tyr-411 leads to **Cys-34**, and Trp-214 leads to **Cys-34** distances were found to be 25.2 +/- 0.6, 25.2 +/- 2.1, and 31.8 +/- 0.8 A, respectively.

L3 ANSWER 36 OF 39 MEDLINE on STN

83231441. PubMed ID: 6860637. Resonance energy transfer between **cysteine-34** and tryptophan-214 in human **serum albumin**. Distance measurements as a function of pH. Suzukida M; Le H P; Shahid F; McPherson R A; Birnbaum E R; Darnall D W. Biochemistry, **(1983 May 10)** 22 (10) 2415-20. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB The single cysteine residue (**Cys-34**) of human **serum albumin** was modified with the organic mercurial [4-[p-(dimethylamino)phenyl]azo]phenyl]mercuric acetate. Introduction of this chromophore into the protein results in the quenching of the protein tryptophan fluorescence spectrum due to energy transfer from the tryptophan residue to the mercurial. Since human albumin contains only a single tryptophan, it was then possible to calculate distances between the mercurial bound at **Cys-34** and Trp-214 under various conditions. This distance contracted during the course of the N leads to F transition, being 34-35 A in the N conformation (pH 6-7.5) and 29.9 A in the F conformation (pH 3.6). The distance increased substantially during the course of the F leads to E transition occurring between pH 3.6 and pH 1.9 and was found to be nearly 37 A at pH 1.9. The distance between **Cys-34** and Trp-214 was found to undergo a slight contraction during the N leads to B transition occurring between pH 7.0 and pH 9.0. At pH 8.5-9 where the protein is predominately in the B form, the distance was found to be slightly more than 31 A.

L3 ANSWER 37 OF 39 MEDLINE on STN

83213547. PubMed ID: 6189837. Complexes of **serum albumin** and cis-dichlorodiammineplatinum (II). The role of **cysteine 34** as a nucleophilic entering group and evidence for reaction between bound platinum and a second macromolecule. Gonias S L; Pizzo S V. Journal of biological chemistry, **(1983 May 10)** 258 (9) 5764-9. Journal code:

2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

L3 ANSWER 38 OF 39 MEDLINE on STN
82239366. PubMed ID: 7096338. The biosynthesis of rat **serum albumin**. In vivo studies on the formation of the disulfide bonds. Peters T Jr; Davidson L K. Journal of biological chemistry, **(1982 Aug 10)** 257 (15) 8847-53. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB In order to learn at what stage the disulfide bonds of albumin are formed during its biosynthesis, we perfused rat livers with iodoacetamide and then isolated the intracellular precursor, proalbumin, from organelles known to be in the pathway of albumin synthesis and secretion. The alkylated cysteines in proalbumin were determined as a measure of its thiol groups in vivo. Proalbumin of smooth microsomes was found to contain a single thiol, which is proposed to be the noncoupling cysteine occurring residue 34 in circulating albumin. Proalbumin in rough microsomes contained an average of two cysteines; the additional cysteine thiol was largely situated in the COOH-terminal region and disappeared rapidly after blocking albumin synthesis with cycloheximide. In nascent chains of proalbumin, about 45% of the cysteine + cystine was in the thiol form. From these findings we propose that disulfide bond formation begins while the nascent chain is still attached to the ribosome and proceeds in an NH2 to COOH direction. The disulfide bonding apparently is completed into the endoplasmic reticulum. Possible intermediates in the process such as mixed disulfide forms of proalbumin with glutathione or cystamine were not detected. We suggest that **cysteine-34** does not participate in disulfide bonding because the NH2 terminus of proalbumin remains loosely bound to the membrane, attached by a hydrophobic segment of the chain at residues 21-27.

L3 ANSWER 39 OF 39 MEDLINE on STN
80156821. PubMed ID: 6244951. Temperature behaviour of human **serum albumin**. Wetzel R; Becker M; Behlke J; Billwitz H; Bohm S; Ebert B; Hamann H; Krumbiegel J; Lassmann G. European journal of biochemistry / FEBS, **(1980 Mar)** 104 (2) 469-78. Journal code: 0107600. ISSN: 0014-2956. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.
AB Structural alterations of albumin, their dependence on concentration and the role of free --SH groups at thermal denaturation, as well as the reversibility of thermally induced structural changes, were studied. Application of various physical methods provides information on a series of structural parameters in a major concentration range. Apart from changes of the helix content, heat treatment gives rise to beta structures which are amplified on cooling and which are correlated with the aggregation of albumin. With rising temperature and concentration the proportion of beta structures and aggregates increases. At degrees of denaturation of up to 20% complete renaturation is possibly in every case. The structure content is concentration-dependent even at room temperature. It may be that intermolecular interactions induce additional alpha-helix structures which are less stable, however, than the ones stabilized by intramolecular interactions. Unfolding of the pocket containing the free --SH group of **cysteine-34** enables disulphide bridges to be formed leading to stable aggregates and irreversible structural alterations. Through binding of N-ethymaleimide to free --SH groups, which blocks the formation of disulphide bridges, it is possible to prevent aggregation and irreversible conformational changes. At temperatures below 65--70 degrees C, oligomers are formed mainly via intermolecular beta structures.

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     (FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

     FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005
L1         68146 S (SERUM ALBUMIN)
L2            74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3            39 S L2 AND PY<2000

=> s l1 and pharmacolog?
       1979072 PHARMACOLOG?
L4       15041 L1 AND PHARMACOLOG?

=> s l4 and (conjugat? or fusion or linked)
         78444 CONJUGAT?
        125944 FUSION
        247085 LINKED
L5        1533 L4 AND (CONJUGAT? OR FUSION OR LINKED)

=> s l5 (increased stability or increased clearance or increased half-life)
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MISSING OPERATOR 'L5 (INCREASED'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l5 and (increased stability or delayed clearance or increased circulation or increased half-life)
1214534 INCREASED
134740 STABILITY
1109 INCREASED STABILITY
(INCREASED(W)STABILITY)
154790 DELAYED
90311 CLEARANCE
332 DELAYED CLEARANCE
(DELAYED(W)CLEARANCE)
1214534 INCREASED
198277 CIRCULATION
60 INCREASED CIRCULATION
(INCREASED(W)CIRCULATION)
1214534 INCREASED
226783 HALF
398797 LIFE
133 INCREASED HALF-LIFE
(INCREASED(W)HALF(W)LIFE)
L6 2 L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED CIRCULATION OR INCREASED HALF-LIFE)

=> d l6,cbib,ab,1-2

L6 ANSWER 1 OF 2 MEDLINE on STN
2001506683. PubMed ID: 11555696. Prolonged in vivo anticoagulant activity of a hirudin-albumin **fusion** protein secreted from Pichia pastoris. Sheffield W P; Smith I J; Syed S; Bhakta V. (Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ont., Canada.. sheffiel@mcmaster.ca) . Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis, (2001 Sep) 12 (6) 433-43. Journal code: 9102551. ISSN: 0957-5235. Pub. country: England: United Kingdom. Language: English.

AB Hirudin is a small, proteinaceous thrombin inhibitor that clears rapidly from the circulation. A hexahistidine-tagged hirudin-rabbit **serum albumin** (RSA) **fusion** protein, HLAH6, was characterized following secretion from Pichia pastoris. HLAH6 bound to immobilized nickel, anti-RSA, and anti-hexahistidine antibodies, and contained the expected (ITYTD) N-terminus. Its spectrometric mass was 74,490 (versus the theoretical mass of 74,410 and sodium dodecyl sulfate-polyacrylamide gel electrophoresis mobility of 84 kDa). The terminal catabolic half-life in rabbits of HLAH6, recombinant Pichia-derived His-tagged RSA, or plasma-derived RSA did not differ. Injection of 2 mg/kg HLAH6 into rabbits raised the activated partial thromboplastin time (aPTT) above initial values for 4-24 h, while the equimolar dose of unfused hirudin was without significant effect. A higher dose of HLAH6 (3 mg/kg functional HLAH6, equivalent to 37.6 thrombin-inhibitory units/g) raised the aPTT by 2.0- to 2.5-fold; the elevation persisted for > 48 h. Importantly, both HLAH6 and unfused hirudin inhibited clot-bound thrombin. Our results suggest that HLAH6 exhibits not only **delayed clearance**, but also prolonged biological activity in vivo compared with unfused hirudin.

L6 ANSWER 2 OF 2 MEDLINE on STN
90167655. PubMed ID: 2306723. Characterization and anticancer activity of the micelle-forming polymeric anticancer drug adriamycin-**conjugated** poly(ethylene glycol)-poly(aspartic acid) block copolymer. Yokoyama M; Miyauchi M; Yamada N; Okano T; Sakurai Y; Kataoka K; Inoue S. (Institute of Biomedical Engineering, Tokyo Women's Medical College, Japan.) Cancer research, (1990 Mar 15) 50 (6) 1693-700. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Adriamycin (ADR), an anthracycline anticancer drug, was bound to the poly(aspartic acid) chain of poly(ethylene glycol)-poly(aspartic acid) block copolymer by amide bond formation between an amino group of Adriamycin and the carboxyl groups of the poly(aspartic acid) chain. The polymeric drug thus obtained was observed to form a micelle structure possessing diameter of approximately 50 nm, with a narrow distribution, in phosphate-buffered saline and to show excellent water solubility despite a large amount of ADR introduction. Further, it was able to be stored in lyophilized form without losing its water solubility in the redissolving procedure. **Increased stability** of the bound Adriamycin molecules in phosphate-buffered saline and elimination of binding affinity for bovine **serum albumin** due to the micelle formation were further advantages of this polymeric drug. In vivo high anticancer activity of this micelle-forming polymeric drug against P 388 mouse leukemia was obtained with less body weight loss than that seen with free ADR, due to low toxicity as compared with free ADR.

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     (FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

     FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005
L1         68146 S (SERUM ALBUMIN)
L2            74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3            39 S L2 AND PY<2000
L4         15041 S L1 AND PHARMACOLOG?
L5          1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6             2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED

=> s l4 and (maleimid?)
           4746 MALEIMID?
L7           47 L4 AND (MALEIMID?)

=> s l7 and py<2000
       12397709 PY<2000
L8           28 L7 AND PY<2000

=> d l8,cbib,ab,1-28

L8   ANSWER 1 OF 28     MEDLINE on STN
1999261936.    PubMed ID: 10330050.   Glycated albumin stimulation of PKC-beta
     activity is linked to increased collagen IV in mesangial cells. Cohen M P;
     Ziyadeh F N; Lautenslager G T; Cohen J A; Shearman C W. (Institute of
     Metabolic Research and Exocell, University City Science Center, University
     of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. ) American journal
     of physiology, (1999 May) 276 (5 Pt 2) F684-90. Journal code: 0370511.
     ISSN: 0002-9513. Pub. country: United States. Language: English.
AB   Albumin modified by Amadori-glucose adducts induces coordinate increases
     in the expression of extracellular matrix proteins, transforming growth
     factor (TGF)-beta1, and the TGF-beta type II receptor in glomerular
     mesangial cells. Because activation of protein kinase C (PKC) accompanies
     the increased mesangial cell expression of matrix proteins and TGF-beta1
     induced by high ambient glucose, we postulated that glycated albumin (GA)
     modulates PKC activity and that PKC participates in mediating the
     GA-induced stimulation of matrix production. To test this hypothesis, we
     examined the effects of PKC inhibitors on collagen type IV production by
     mouse or rat mesangial cells incubated with GA, and the influence of GA on
     PKC activity in these cells. Increased collagen type IV production evoked
     by GA in 5.5 and 25 mM glucose in mouse mesangial cells was prevented by
     both general (GF-109203X) and beta-specific (LY-379196) PKC inhibitors.
     Total PKC activity, measured by phosphorylation of a PKC-specific
     substrate, increased with time after exposure of rat mesangial cells to GA
     compared with the nonglycated, glucose-free counterpart. GA caused an
     increase in PKC-beta1 membrane-bound fraction and in total PKC activity in
     media containing physiological (5.5 mM) glucose concentrations in rat
     mesangial cells, confirming that the glucose-modified protein, and not a
     "hyperglycemic" milieu, was responsible. The findings indicate that
     Amadori-modified albumin stimulates mesangial cell PKC activity, and that
     activation of the PKC-beta isoform is linked to the stimulation of
     collagen type IV production.

L8   ANSWER 2 OF 28     MEDLINE on STN
1999008560.    PubMed ID: 9794429.   Inhibition by dexamethasone of
     antigen-induced c-Jun N-terminal kinase activation in rat basophilic
     leukemia cells. Hirasawa N; Sato Y; Fujita Y; Mue S; Ohuchi K. (Department
     of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences,
     Tohoku University, Sendai, Japan.. hirasawa@mail.pharm.tohoku.ac.jp) .
     Journal of immunology (Baltimore, Md. : 1950), (1998 Nov 1) 161 (9)
     4939-43. Journal code: 2985117R. ISSN: 0022-1767. Pub. country: United
     States. Language: English.
AB   Antigen stimulation of IgE-sensitized rat basophilic leukemia RBL-2H3
     cells induced activation of c-Jun N-terminal kinase (JNK) within a few
     minutes with maximum activity attained 40 min later. The increase in JNK
     activity was accompanied with an increase in phosphorylation of c-Jun in
     the cells. The Ag-induced JNK activation was inhibited by the
     phosphatidylinositol 3-kinase inhibitors wortmannin (10-100 nM) and LY
     294002 (100 microM) but not by the protein kinase C inhibitors calphostin
     C (1 and 3 microM) and Ro 31-8425 (1 and 3 microM). Pretreatment with
     dexamethasone (10 and 100 nM) for 18 h inhibited the Ag-induced increase
     in JNK activity in a concentration-dependent manner. At least 6 h of
     preincubation with dexamethasone was necessary to inhibit the Ag-induced
     JNK activation. The phosphorylation of c-Jun induced by the Ag
     stimulation was reduced by pretreatment with dexamethasone without
     reduction of the content of c-Jun protein. The Ag-induced activation of
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the JNK kinase kinase mitogen-activated protein kinase-extracellular signal-regulated kinase kinase-1 was also inhibited by pretreatment with dexamethasone at 10 and 100 nM. These findings indicate that dexamethasone reduces JNK protein level and inhibits the Ag-induced activation of JNK resulting in the inhibition of c-Jun phosphorylation.

L8 ANSWER 3 OF 28 MEDLINE on STN
1998185800. PubMed ID: 9525088. Albumin conjugates of the anticancer drug chlorambucil: synthesis, characterization, and in vitro efficacy. Kratz F; Beyer U; Roth T; Schutte M T; Unold A; Fiebig H H; Unger C. (Tumor Biology Center, Dept. Med. Oncology, Freiburg, Germany.) Archiv der Pharmazie, **(1998 Feb)** 331 (2) 47-53. Journal code: 0330167. ISSN: 0365-6233. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.
AB In our efforts to improve the selectivity and toxicity profile of antitumor agents, four **maleimide** derivatives of chlorambucil (1-4) were bound to thiolated human **serum albumin** which differ in the stability of the chemical link between drug and spacer. 1 is an aliphatic **maleimide** ester derivative of chlorambucil, whereas 2-4 are acetaldehyde, acetophenone, and benzaldehyde carboxylic hydrazone derivatives. HPLC stability studies at pH 5.0 with the related model compounds 5, 7, 8, and 9, in which chlorambucil was substituted by 4-phenylbutyric acid, demonstrated that the carboxylic hydrazone derivatives have acid-sensitive properties; the acid lability of 7 was particular prominent with a half-life of only a few hours. The alkylating activity of albumin-bound chlorambucil was determined with the aid of 4-(4-nitrobenzyl)-pyridine (NBP), demonstrating that on average three equivalents were protein-bound. Evaluation of the cytotoxicity of free chlorambucil and the respective albumin conjugates in the MCF7 mamma carcinoma and MOLT4 leukemia cell line employing a propidium iodide fluorescence assay demonstrated that the conjugate in which chlorambucil was bound to albumin through an ester bond was not as active as chlorambucil. In contrast, the conjugates in which chlorambucil was bound to albumin through carboxylic hydrazone bonds were as or more active than chlorambucil in both cell lines. In particular, the conjugate in which chlorambucil was bound to albumin through an acetaldehyde carboxylic hydrazone bond exhibited IC50 values which were approximately 4-fold (MCF7) to 13-fold (MOLT4) lower than those of chlorambucil. Preliminary toxicity studies in mice showed that this conjugate can be administered at higher doses in comparison to unbound chlorambucil.

L8 ANSWER 4 OF 28 MEDLINE on STN
1998136036. PubMed ID: 9477169. Preparation, characterization and in vitro efficacy of albumin conjugates of doxorubicin. Kratz F; Beyer U; Collery P; Lechenault F; Cazabat A; Schumacher P; Falken U; Unger C. (Tumor Biology Center, Department of Medical Oncology, Clinical Research, Freiburg, Federal Republic of Germany.) Biological & pharmaceutical bulletin, **(1998 Jan)** 21 (1) 56-61. Journal code: 9311984. ISSN: 0918-6158. Pub. country: Japan. Language: English.
AB One strategy for improving the antitumor selectivity and toxicity profile of antitumor agents is to design drug carrier systems with suitable transport proteins. Thus, four **maleimide** derivatives of doxorubicin were bound to thiolated human **serum albumin** which differed in the stability of the chemical link between drug and spacer. In the **maleimide** derivatives, 3-**maleimidobenzoic** or 4-**maleimidophenylacetic** acid was bound to the 3'-amino position of doxorubicin through a benzoyl or phenylacetyl amide bond and 3-**maleimidobenzoic** acid hydrazide or 4-**maleimidophenylacetic** acid hydrazide was bound to the 13-keto position through a benzoyl hydrazone or phenylacetyl hydrazone bond. The acid-sensitive albumin conjugates prepared with the carboxylic hydrazone doxorubicin derivatives exhibited an inhibitory efficacy in the MDA-MB-468 breast cancer cell line and U937 leukemia cell line comparable with that of the free drug (using the BrdU-(5-bromo-2'-deoxyuridine)-incorporation assay and tritiated thymidine incorporation assay respectively, IC50 approximately 0.1-1 microM) whereas conjugates with the amide derivatives showed no or only marginal activity. These results demonstrate that antiproliferative activity depends on the nature of the chemical bond between doxorubicin and carrier protein. Acid-sensitive albumin conjugates are suitable candidates for further in vitro and in vivo assessment.

L8 ANSWER 5 OF 28 MEDLINE on STN
97423533. PubMed ID: 9277475. Vinculin phosphorylation and barrier failure of coronary endothelial monolayers under energy depletion. Muhs A; Noll T; Piper H M. (Physiologisches Institut, Justus-Liebig-Universitat, Giessen, Germany.) American journal of physiology, **(1997 Aug)** 273 (2 Pt 2) H608-17. Journal code: 0370511. ISSN: 0002-9513. Pub. country: United States. Language: English.
AB We studied the hypothesis that, in energy-depleted endothelial cells, Ca(2+)-dependent activation of protein kinase C (PKC) causes

phosphorylation of vinculin and that this effect is involved in the early loss of endothelial barrier function. Vinculin localization and phosphorylation, PKC activity, and albumin permeability were studied in cultured coronary endothelial monolayers from rats. Ten minutes after the onset of metabolic inhibition by 5 mM potassium cyanide and 5 mM 2-deoxy-D-glucose, immunofluorescence of vinculin at cell-to-cell and cell-to-matrix contacts faded, whereas total cellular vinculin content remained unchanged. During the same time period, vinculin phosphorylation at tyrosine and serine sites increased by 3.9- and 3.5-fold, respectively. Vinculin phosphorylation was related to activation of PKC and an unidentified tyrosine kinase and was elicited by a rise in cytosolic Ca2+ within energy-depleted endothelial cells. Conditions inhibiting vinculin phosphorylation also reduced monolayer permeability induced by energy depletion. These data indicate that vinculin phosphorylation is involved in the progression of hyperpermeability during energy depletion in coronary endothelial monolayers.

L8 ANSWER 6 OF 28 MEDLINE on STN

97361240. PubMed ID: 9218134. Negative regulation of MAP kinase by diacylglycerol-dependent mechanisms via G protein-coupled receptors in rat basophilic RBL-2H3 (ml) cells. Hirasawa N; Mue S; Ohuchi K. (Department of Pathophysiological Biochemistry, Faculty of Pharmaceutical Sciences, Tohoku University Sendai, Miyagi, Japan.) Cellular signalling, **(1997 May-Jun)** 9 (3-4) 319-22. Journal code: 8904683. ISSN: 0898-6568. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Carbachol and 5'-(N-ethylcarboxamido)-adenosine (NECA), stimulants of G protein-coupled receptors, induce MAP kinase activation in the muscarinic ml receptor-transfected mast cell line, RBL-2H3 (ml) cells. The phospholipase C inhibitor neomycin and the phosphatidate phosphohydrolase inhibitor propranolol augmented MAP kinase activation induced by carbachol and NECA without affecting the antigen-induced MAP kinase activation. Furthermore, the duration of MAP kinase activation induced by carbachol or NECA was also prolonged by neomycin and propranolol. The specific protein kinase C inhibitor Ro 31-8425 enhanced the carbachol- or NECA-induced MAP kinase activation. These findings suggest that the MAP kinase activation mediated by the G protein-coupled receptors is negatively regulated by diacylglycerol and activated protein kinase C(s).

L8 ANSWER 7 OF 28 MEDLINE on STN

97302612. PubMed ID: 9158859. Covalent linkage of recombinant hirudin to poly(ethylene terephthalate) (Dacron): creation of a novel antithrombin surface. Phaneuf M D; Berceli S A; Bide M J; Quist W C; LoGerfo F W. (Deaconess Hospital/Harvard Medical School, Vascular Surgery Research, Boston, MA 02215, USA.) Biomaterials, **(1997 May)** 18 (10) 755-65. Journal code: 8100316. ISSN: 0142-9612. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Thrombus formation and intimal hyperplasia on the surface of implantable biomaterials such as poly(ethylene terepthalate) (Dacron) vascular grafts are major concerns when utilizing these materials in the clinical setting. Thrombin, a pivotal enzyme in the blood coagulation cascade primarily responsible for thrombus formation and smooth muscle cell activation, has been the target of numerous strategies to prevent this phenomenon from occurring. The purpose of this study was to covalently immobilize the potent, specific antithrombin agent recombinant hirudin (rHir) to a modified Dacron surface and characterize the in vitro efficacy of thrombin inhibition by this novel biomaterial surface. Bovine **serum albumin** (BSA), which was selected as the "basecoat' protein, was reacted with various molar ratios of the cross-linker sulphosuccinimidyl 4-(N-**maleimidomethyl**) cyclohexane-1-carboxylate (sulpho-SMCC; 1:5-1:50). These BSA-SMCC complexes were then covalently linked to sodium hydroxide-hydrolysed Dacron (HD) segments via the cross-linker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). Covalent linkage of these complexes to HD (HD-BSA-SMCC) was not affected by any of the sulpho-SMCC cross-linker ratios assayed. rHir, which was initially reacted with 2-iminothiolane hydrochloride (Traut's reagent) in order to create sulphydryl groups, was then covalently bound to these HD-BSA-SMCC surfaces (HD-BSA-SMCC-S-rHir). The 1:50 (BSA: sulpho-SMCC) HD-BSA-SMCC-S-rHir segments bound 22-fold more rHir (111 ng per mg Dacron) compared to control segments and also possessed the greatest thrombin inhibition of the segments evaluated using a chromogenic substrate assay for thrombin. Further characterization of the HD-BSA-SMCC-S-rHir segments demonstrated that maximum thrombin inhibition was 20.43 NIHU, 14.6-fold greater inhibition than control segments (1.4 NIHU). Thrombin inhibition results were confirmed by 125I-thrombin binding experiments, which demonstrated that the 1:50 HD-BSA-SMCC-S-rHir segments had significantly greater specific thrombin adhesion compared to control segments. Non-specific 125I-thrombin binding to and release from the 1:50 HD-BSA-SMCC-S-rHir segments was also significantly less than the control segments. Thus, these results demonstrate that rHir can be covalently

bound to a clinically utilized biomaterial (Dacron) while still maintaining its ability to bind and inhibit thrombin.

L8 ANSWER 8 OF 28 MEDLINE on STN

97102715. PubMed ID: 8946955. Quantitative analysis of exocytosis visualized by a video-enhanced light/fluorescence microscope reveals two distinct components of exocytosis in RBL-2H3 cells. Ozawa K; Kobayashi H; Kawai E; Suzaki E; Nonomura Y; Masujima T. (Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Japan.) FEBS letters, **(1996 Nov 25)** 398 (1) 67-73. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Rat basophilic leukemia (RBL-2H3) cells, which exhibit Ca2+-dependent secretion of granules when stimulated with antigen or the Ca2+-ionophore A23187, were observed under a video-enhanced light/fluorescence microscope. Exocytotic events of individual granules were visualized in individual cells stimulated with antigen or A23187. Exocytosis of granules stimulated with A23187 showed two peaks in the time course. The earlier one was inhibited by selective inhibitors of protein kinase C (Ro31-8425, Ro31-8220, and chelerythrine) and the other was inhibited by an inhibitor of phosphatidate hydrolase, propranolol. Exocytosis by antigen stimulation, however, showed only one peak, which was inhibited by the selective inhibitors of protein kinase C, but not by propranolol. These results indicate that at least two distinct components of exocytosis exist in RBL-2H3 cells.

L8 ANSWER 9 OF 28 MEDLINE on STN

97027829. PubMed ID: 9125277. Interaction of glucose and metformin with isolated red cell membrane. Freisleben H J; Furstenberger H J; Deisinger S; Freisleben K B; Wiernsperger N; Zimmer G. (Gustav-Embden-Zentrum der Biologischen Chemie, Klinikum der Johann Wolfgang Goethe-Universitat, Frankfurt/Main, Germany.) Arzneimittel-Forschung, **(1996 Aug)** 46 (8) 773-8. Journal code: 0372660. ISSN: 0004-4172. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Isolated human erythrocyte membranes (red blood cell (RBC) ghosts) were incubated with glucose at 5, 10, 20 and 100 mmol/l concentrations, with insulin (0.01 to 200 mU/l) and metformin (CAS 657-24-9) 0.5 up to 50.0 mumol/l). Binding studies with 14C-glucose and subsequent gel electrophoresis revealed 60% of the radioactivity around ban 4.2-4.5 at 5 mmol/l, whereas a random distribution of radioactivity over all protein bands of the RBC membrane was found at 20 mmol/l concentration after incubation for 30 min or 48 h. Metformin does not bind covalently to RBC membranes, however, after photochemical linkage of 14C-metformin via the aminoreactive linker azidophenylglyoxal the highest radioactivity (21%) was counted in the range of band 4.2-4.5. In parallel with an increase of order parameters of 5-doxyl-stearic acid the thiol status of the membranes decreases as determined by monobromobimane fluorescence. 20 and 100 mmol/l concentrations of glucose decrease the reactivity of membrane thiols towards bromobimane significantly to 73 and 62% of the controls. Concomitantly, membrane fluidity at polar sites is diminished as measured by order parameters of spin label 5-doxyl stearic acid. In RBC membranes pretreated with 20 mmol/l glucose the decreased fluorescence is significantly raised again by insulin and metformin. This effect is even more pronounced, if insulin and metformin are incubated together. Reaction of membrane thiols with a **maleimido** spin label detects modification in the ratio of mobile and immobilized spin label populations in the electron paramagnetic resonance signal under the above conditions, indicative of conformational changes of membrane proteins.

L8 ANSWER 10 OF 28 MEDLINE on STN

96234679. PubMed ID: 8642195. Immunogenicity of new heterobifunctional cross-linking reagents used in the conjugation of synthetic peptides to liposomes. Boeckler C; Frisch B; Muller S; Schuber F. (Laboratoire de Chimie Bioorganique, CNRS URA 1386, Faculte de Pharmacie, Illkirch, France.) Journal of immunological methods, **(1996 May 10)** 191 (1) 1-10. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB We have investigated the immunogenicity of six thiol-reactive heterobifunctional cross-linking reagents that permit the conjugation of cysteine carrying peptides to the surface of liposome containing monophosphoryl lipid A. Such constructs elicit an immune response against short synthetic peptides and our aim was to find the least immunogenic linkers to limit potential carrier-induced epitopic suppression. For that purpose the properties of three new polyoxyethylene linkers of different lengths and thiol-reactive moieties (**maleimide**, bromoacetyl, dithiopyridine) were compared to known derivatives obtained by reacting the classical reagents SMPB and SPDP or N-succinimidyl bromoacetate with phosphatidylethanolamine. The least immunogenic linkers were the bromoacetate derivatives whereas those containing a **maleimide** group evoked a significant anti-linker immune response. In addition, using

IRGERA as a model peptide, we found that all six liposomal constructs strongly elicited the production of anti-peptide IgG antibodies. This immune response was therefore independent of the length of the linkers (ranging between 0.3 and 1.6 nm) and of the nature of the linkage. between the peptide and the thiol-reactive moieties of the cross-linkers, i.e. stable thioether or bio-reducible disulfide bonds.

L8 ANSWER 11 OF 28 MEDLINE on STN

96174859. PubMed ID: 8593275. Preparation and characterization of conjugates of (modified) human **serum albumin** and liposomes: drug carriers with an intrinsic anti-HIV activity. Kamps J A; Swart P J; Morselt H W; Pauwels R; De Bethune M P; De Clercq E; Meijer D K; Scherphof G L. (Groningen Institute for Drug Studies, Department of Physiological Chemistry, Groningen University, Groningen, The Netherlands.) Biochimica et biophysica acta, **(1996 Jan 31)** 1278 (2) 183-90. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Human **serum albumin** (HSA) derivatized with cis-aconitic anhydride (Aco-HSA) that was earlier shown to inhibit replication of human immunodeficiency virus type 1 (HIV-1), was covalently coupled to conventional liposomes, consisting of phosphatidylcholine, cholesterol and **maleimido**-4-(p-phenylbutyryl)phosphatidylethanolamine, using the heterobifunctional reagent N-succinimidyl-S-acetylthioacetate (SATA). The amount of HSA that could be coupled to the liposomes depended on derivatization of the HSA and ranged from 64.2 +/- microgram HSA/micromol total lipid for native HSA to 29.5 +/- 2.7 microgram HSA/micromol total lipid for HSA in which 53 of the epsilon amino groups of lysine were derivatized with cis-aconitic anhydride (Aco53-HSA). Incorporation of 3.8 mol% of total lipid of a poly(ethylene glycol) derivative of phosphatidylethanolamine (PEG-PE) in the liposomes resulted in a lower coupling efficiency of Aco-HSA. The elimination and distribution of the liposomal conjugates in rats in vivo was largely dependent on the modification of the HSA coupled to the liposomes. With native HSA-liposomes, more than 70% of the conjugate was still found in the blood plasma 30 min after i.v. injection in rats, while at this time Aco-HSA-liposomes were completely cleared from the circulation. The rapid clearance of conventional Aco-HSA-liposomes was due to a rapid uptake into the liver and could be considerably decreased by incorporating PEG-PE in the liposomal bilayer. After 3 h 60% of Aco-HSA-PEG-liposome conjugates were found in the blood. In an in vitro anti-HIV-1 assay, the 50% inhibitory concentrations (IC50) for Aco39-HSA-liposomes and Aco53-HSA-liposomes expressed as protein weight, were 2.87 microgram/ml and 0.154 microgram/ml, respectively. When PEG-PE was incorporated, the Aco53-HSA-liposomes retained anti HIV-1 activity (IC50:3.13 microgram/ml). The possibility to modulate the residence time in the bloodstream of Aco-HSA-liposomes and the potent anti-HIV-1 activity of these conjugates, may allow the development of an intrinsically active drug carrier system. By incorporating anti HIV-1 drugs such as AZT into such liposomes a drug delivery system can be designed that might act simultaneously on the virus/cell binding by virtue of the coupled Aco-HSA and on the RNA/DNA transcription of the HIV-1 replication cycle through the nucleoside analogue.

L8 ANSWER 12 OF 28 MEDLINE on STN

94170835. PubMed ID: 8125132. Effect of a selective protein kinase C inhibitor, Ro 31-8425, on Mac-1 expression and adhesion of human neutrophils. Sullivan J A; Merritt J E; Budd J M; Booth R F; Hallam T J. (Roche Research Centre, Welwyn Garden City, GB.) European journal of immunology, **(1994 Mar)** 24 (3) 621-6. Journal code: 1273201. ISSN: 0014-2980. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB The role of protein kinase C (PKC) in mediating up-regulation of macrophage 1 adhesion protein (Mac-1) and adhesion of neutrophils in response to physiological agonists is not clear. Previous studies have relied on use of phorbol esters to activate PKC directly or on results obtained with non-selective inhibitors of protein kinases. 3-[8-(Aminomethyl)-6,7,8,9-tetrahydropyridol[1,2-a]-indol-10-yl]-4 -(1-methyl-3-indolyl)-1H-pyrrole-2,5-dione hydrochloride (Ro 31-8425) is a potent and highly selective inhibitor of PKC (Bit et al. J. Med. Chem. 1993. 36: 21). In these studies Ro 31-8425 has been used to define, more definitively, the role of PKC in mediating complement fragment C5a (C5a)-stimulated up-regulation of Mac-1 and adhesion of neutrophils to endothelial cells and to bovine **serum albumin** (BSA)-coated plastic. Phorbol 12, 13 dibutyrate (PBu2) increased surface expression of Mac-1 and stimulated adhesion of neutrophils to endothelial cells and to BSA-coated plastic. This confirms previous reports that activation of PKC can stimulate these responses. The PKC inhibitor, Ro 31-8425, inhibited the PBu2-stimulated responses, which confirms that Ro 31-8425 was effective in inhibiting PKC in these neutrophils. A more physiological agonist, C5a, also increased surface expression of Mac-1 and adhesion of neutrophils to

endothelial cells and BSA-coated plastic. However, the responses to C5a were unaffected by Ro 31-8425. These results suggest that, although activation of PKC can promote up-regulation of Mac-1 and adhesion of neutrophils, this does not appear to be the physiological pathway. A non-selective protein kinase inhibitor, staurosporine, inhibited both PBu2 and C5a-stimulated adhesion. This suggests that a protein kinase other than PKC, possibly a tyrosine protein kinase, is likely to be involved in mediating C5a-stimulated Mac-1 up-regulation and adhesion. These results emphasise the need for caution in interpreting experiments and assuming a role for PKC. Use of a potent and selective inhibitor of PKC, Ro 31-8425, provides more definitive information.

L8 ANSWER 13 OF 28 MEDLINE on STN

94012853. PubMed ID: 8408112. Detection of remnant proteolytic activities in unimplanted glutaraldehyde-treated bovine pericardium and explanted cardiac bioprostheses. Simionescu D; Simionescu A; Deac R. (Cardiovascular Surgery Research Department, Public Health and Medical Research Institute, Tirgu Mures, Romania.) Journal of biomedical materials research, **(1993 Jun)** 27 (6) 821-9. Journal code: 0112726. ISSN: 0021-9304. Pub. country: United States. Language: English.

AB The presence and activity of proteolytic enzymes has been investigated in vitro on soluble and insoluble preparations obtained from both unimplanted and implanted glutaraldehyde-treated bovine parietal pericardium. Using detection by colorimetric techniques, soluble preparations were shown to hydrolyze enzyme substrates that are characteristic for trypsin-like proteases, cathepsin-like proteases, and collagenase. As detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in gradient gels and gel filtration on Sepharose CL-6B, insoluble (pellet) preparations degraded denatured type I collagen in a time-dependent pattern, producing low-molecular-weight fragments. These activities were partially inhibited by phenylmethylsulfonyl fluoride, N-ethyl **maleimide**, soybean trypsin inhibitor, para-chloromercuribenzoic acid, or ethylenediaminetetraacetic acid, suggesting the presence of a heterogeneous enzymatic mixture. Insoluble preparations incubated with pure pericardial dermatan sulfate proteoglycan detached the glycosaminoglycan chains from their core protein carrier, producing a digestion pattern similar to Cathepsin C. These findings demonstrate the presence of active proteases in glutaraldehyde-fixed bovine pericardium per se and in explanted pericardial bioprosthetic cardiac valves, an additional factor that might contribute to intrinsic extracellular matrix degeneration in pericardial bioprosthetic devices.

L8 ANSWER 14 OF 28 MEDLINE on STN

93119500. PubMed ID: 1476657. Immunomodulating activity of 1,2-difattyacyl-3-mercaptoglycerol adducts. Gemeiner M; Leidinger E; Miller I; Moroder L. (Institut fur Medizinische Chemie, Veterinarmedizinische Universitat Wien.) Biological chemistry Hoppe-Seyler, **(1992 Nov)** 373 (11) 1085-94. Journal code: 8503054. ISSN: 0177-3593. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Adducts of 1,2-diacyl-(2RS)-3-mercaptoglycerol to 3-**maleimidopropionic** acid and to 3-**maleimidopropionyl**-L-alanyl-D-isoglutamine as well as a new lipophilic MTP derivative have been analysed for their immunomodulating activity in comparison to the well known adjuvants CFA and IFA using a suboptimal concentration of BSA as antigen. A concentration-dependent immunostimulating activity was established for these lipophilic molecules when applied in water/oil (IFA) emulsions. The results clearly indicate that the new series of compounds behave as competent adjuvants, a fact which is further supported by the immunoadjuvant effect triggered by two of these diacylthioglyceryl adducts as examples, even when applied in aqueous systems. In the light of these findings it seems promising to use these thiol-functionalized molecules to build in immunoadjuvancy via the **maleimide**-thiol reaction principle. Attachment of such "sticky fingers" to peptides might enhance interaction of haptens and immunogens with cell membranes and thus, may represent a useful tool for inducing or increasing immunological responses to synthetic antigen presenting systems.

L8 ANSWER 15 OF 28 MEDLINE on STN

91064842. PubMed ID: 2123420. A highly sensitive enzyme-linked immunosorbent assay for etoposide using beta-D-galactosidase as a label. Saita T; Fujiwara K; Kitagawa T; Mori M; Takata K. (Faculty of Hospital Pharmacy, Saga Medical School, Japan.) Cancer chemotherapy and pharmacology, **(1990)** 27 (2) 115-20. Journal code: 7806519. ISSN: 0344-5704. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB A highly sensitive enzyme-linked immunosorbent assay (ELISA) for etoposide (EP) was developed, which is capable of accurately measuring as little as 40 pg EP/ml. Anti-EP sera were obtained by immunizing rabbits with EP

conjugated with mercaptosuccinyl bovine **serum albumin** (MS.BSA) using N-[beta-(4-diazophenyl)ethyl]**maleimide** (DPEM) as a heterobifunctional coupling agent. An enzyme marker was similarly prepared by coupling EP with beta-D-galactosidase (beta-Gal; EC 3.2.23) via DPEM. This ELISA was specific for EP and showed a very slight cross-reactivity with its major metabolite, cis-hydroxy acid of EP (0.91%), but none with 4'-demethylepipodophyllotoxin and drugs commonly used with EP in combination chemotherapy for cancer treatment. The values for EP concentration detected by this assay were comparable with those detected by the high-performance liquid chromatography (HPLC) method. However, the ELISA was about 1,250 times more sensitive in detecting EP at lower concentrations. Using this assay, drug levels were easily determined in the blood and urine of rats for 7 h after i.v. administration of EP at a single dose of 3 mg/kg. Due to its sensitivity and specificity for EP, the ELISA should prove to be a valuable new tool for use in clinical **pharmacological** studies.

L8 ANSWER 16 OF 28 MEDLINE on STN

90001218. PubMed ID: 2790016. Synthesis of a superoxide dismutase derivative that circulates bound to albumin and accumulates in tissues whose pH is decreased. Inoue M; Ebashi I; Watanabe N; Morino Y. (Department of Biochemistry, Kumamoto University Medical School, Japan.) Biochemistry, **(1989 Aug 8)** 28 (16) 6619-24. Journal code: 0370623. ISSN: 0006-2960. Pub. country: United States. Language: English.

AB Protection of tissues from oxidative stress is one of the major prerequisites for aerobic life. Since intravenously injected Cu2+/Zn2+-type superoxide dismutase (SOD) disappears from the circulation with a short half-life of 5 min, its clinical use as a scavenger for superoxide radical is limited. We synthesized a human erythrocyte type SOD derivative (SM-SOD) by linking 2 mol of hydrophobic organic anion, alpha-4-[(6-(N-**maleimido**)hexanoyloxymethyl]cumyl]half-butyl-esterified poly(styrene-co-maleic acid) (SM), to the cysteinyl residues of the dimeric enzyme without decreasing enzymic activity. SM-SOD, but not SOD, bound to an albumin-Sepharose column; the bound SM-SOD was eluted by a buffer solution containing 0.5% sodium dodecyl sulfate or 10 mM warfarin, suggesting that SM-SOD reversibly binds to the warfarin site on albumin. Due to the amphipathic nature of the SMI moiety, SM-SOD bound also to cell membranes particularly when the pH was decreased. In vivo analysis in the rat revealed that intravenously injected SM-SOD circulated bound to albumin with a half-life of 6 h. Postischemic reperfusion arrhythmias were almost completely prevented by a single dose of SM-SOD, but not SOD. Thus, the prolonged half-life of SM-SOD in the circulation and its preferential accumulation in an injured site with decreased pH appeared to be responsible for preventing myocardial injury. These results suggest that superoxide radical and/or its metabolite(s) would play an important role in the pathogenesis of postischemic reperfusion arrhythmias and that SM-SOD may be useful for decreasing tissue injury in ischemic heart disease.

L8 ANSWER 17 OF 28 MEDLINE on STN

87178908. PubMed ID: 2951892. The plasmin heavy chain-urokinase conjugate: a' specific thrombolytic agent. Nakayama Y; Shinohara M; Tani T; Kawaguchi T; Furuta T; Izawa T; Kaise H; Miyazaki W; Nakano Y. Thrombosis and haemostasis, **(1986 Dec 15)** 56 (3) 364-70. Journal code: 7608063. ISSN: 0340-6245. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB Low molecular weight urokinase (LMW-UK) was coupled to the heavy chain of plasmin to make it able to bind to fibrin. The purified conjugate (PHC-UK conjugate), which consisted of equimolar concentrations of each starting material had a molecular weight of 93,600, bound tightly to fibrin-monomer-Sepharose and was not washed off with 1 M NaCl, but was eluted specifically with epsilon-amino caproic acid. The conjugate showed higher fibrinolytic activity than HMW-UK. A control conjugate prepared by coupling human **serum albumin** to LMW-UK (HSA-UK conjugate) showed the same fibrinolytic activity as HMW-UK. The half-lives of these two conjugates in rabbits were about 3 times that of HMW-UK. In an experimental pulmonary embolism model in rabbits, the PHC-UK conjugate showed about 10 times higher thrombolytic activity than HMW-UK, while the HSA-UK conjugate showed similar thrombolytic activity as HMW-UK, and moreover caused severe systemic fibrinogen breakdown. Thus the significant increase in thrombolytic activity after injection of PHC-UK conjugate into rabbits may be due to its newly acquired fibrin binding activity, and not to increase in its half-life. It is concluded that the PHC-UK conjugate may be useful in treatment of thrombosis.

L8 ANSWER 18 OF 28 MEDLINE on STN

87102650. PubMed ID: 3802092. In vitro cytotoxicity of a human **serum albumin**-mediated conjugate of methotrexate with anti-MM46 monoclonal antibody. Endo N; Kato Y; Takeda Y; Saito M; Umemoto N; Kishida K; Hara T.

Cancer research, **(1987 Feb 15)** 47 (4) 1076-80. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB In studies on antitumor antibody:drug conjugates as potential antitumor agents, methotrexate (MTX) was conjugated with a murine monoclonal antibody (aMM46) to an antigen on ascitic mouse mammary tumor MM46 cells (MM antigen) with human **serum albumin** (HSA) as an intermediary. MTX was linked to HSA which had been conditioned to have about 1 mol of thiol group per mol of HSA by dithiothreitol treatment followed by oxidation on standing at 4 degrees C. The MTX linking was performed, without protection of the thiol group of HSA, by using MTX N-succinimidyl ester prepared via MTX intramolecular anhydride. The resulting HSA:MTX was reacted with the immunoglobulin with the **maleimide** group introduced. The aMM46:HSA:MTX obtained retained both antibody binding and drug activities. The cytotoxicity of aMM46:HSA:MTX against MM antigen-positive MM46 cells was greater than that of control 96.5 (anti-human melanoma-associated antigen, p97):HSA:MTX and was inhibited by unconjugated aMM46. No different cytotoxicity of aMM46:HSA:MTX compared with that of 96.5:HSA:MTX was observed against MM antigen-negative mouse mammary tumor MM48 cells. The presence of ammonium chloride or leupeptin abrogated the selective cytotoxicity against MM46 cells of aMM46 conjugate but did not affect the nonspecific cytotoxicity of 96.5:HSA:MTX. These results support the idea that the selective cytotoxicity of aMM46:HSA:MTX is antibody directed and exhibited through lysosomal degradation of the conjugate.

L8 ANSWER 19 OF 28 MEDLINE on STN

86026321. PubMed ID: 2996599. ESR spectral changes induced by chlorpromazine in spin-labeled erythrocyte ghost membranes. Yamaguchi T; Watanabe S; Kimoto E. Biochimica et biophysica acta, **(1985 Nov 7)** 820 (2) 157-64. Journal code: 0217513. ISSN: 0006-3002. Pub. country: Netherlands. Language: English.

AB Chlorpromazine interacted preferentially with membrane proteins rather than membrane lipids in the initial incorporation into human erythrocyte ghosts, as demonstrated by means of the fluorescence quenching and a **maleimide** spin label. In this state the membrane fluidity increased. At higher concentrations of chlorpromazine, the membrane fluidity decreased and a motionally restricted signal from fatty acid spin labels appeared predominantly. However, no such signal appeared in protein-free vesicles. The temperature and pH dependences of the outer hyperfine splitting of this restricted signal were very similar to those of bovine **serum albumin**. On the basis of sodium dodecyl sulfate-polyacrylamide gel electrophoresis of chlorpromazine-treated and -untreated ghosts, it was found that there was no significant difference in membrane proteins between both samples except for the changes of a few bands which were not directly concerned with the occurrence of this restricted signal. These results suggest that the fatty acid spin labels bind preferably to membrane proteins as the lipid domain becomes packed with chlorpromazine.

L8 ANSWER 20 OF 28 MEDLINE on STN

84291236. PubMed ID: 6470624. Synthesis and evaluation of luminescent tracers and hapten-protein conjugates for use in luminescence immunoassays with immobilised antibodies and antigens. A critical study of macro solid phases for use in immunoassay systems, Part II. Gadow A; Fricke H; Strasburger C J; Wood W G. Journal of clinical chemistry and clinical biochemistry. Zeitschrift fur klinische Chemie und klinische Biochemie, **(1984 May)** 22 (5) 337-47. Journal code: 7701860. ISSN: 0340-076X. Pub. country: GERMANY, WEST: Germany, Federal Republic of. Language: English.

AB This article describes the synthesis of labels and hapten-protein conjugates for use in bio- and chemiluminescent immunoassay systems, together with the problems encountered. The effects of **maleimide** upon acetate-, adenylate- and pyruvate kinase activity have been studied, as well as upon the luciferin-luciferase monitoring system. **Maleimide** inhibited both acetate and adenylate kinase but showed no inhibition of pyruvate kinase and the monitoring reagent. Four heterobifunctional reagents were tested for their capability in forming pyruvate kinase-donkey-anti-rabbit IgG conjugates which retained enzyme and antibody activity. The best results were obtained with succinimidyl-4-(N-**maleimidomethyl**)-cyclohexane-1-carboxylate and succinimidyl-6-(p-**maleimidophenyl**)-hexanoate. The relationship between the amounts of succinimidyl-4-(N-**maleimidomethyl**)-cyclohexane-1-carboxylate and IgG was studied with respect to enzymic activity of the conjugate. The Michaelis-Menten constants for both conjugated and non-conjugated pyruvate kinase were calculated and compared. It was found that the maximal velocity (Vmax) of the conjugated enzyme was lower than that of the non-conjugated enzyme although the "apparent" Km value was the same for both conjugated and non-conjugated pyruvate kinase. The pyruvate kinase-anti rabbit IgG conjugate was tested for its ability to bind to rabbit-IgG coated polystyrene balls. In addition to bioluminescent labels, the synthesis of chemiluminescent markers was undertaken and

optimised. The three substances used for labelling were diazoluminol, diazoisoluminol and N-(4-aminobutyl)-N-ethylisoluminol hemisuccinamide the latter being used as an N-hydroxysuccinamide "active" ester. The ratio of label to IgG was studied for diazoluminol and N-(4-aminobutyl)-N-ethylisoluminol hemisuccinamide active ester after it had been discovered that diazoisoluminol was not suitable for coupling to antibodies. The optimal molar ratios label: IgG were for diazoluminol 40:1 and for N-(4-aminobutyl)-N-ethylisoluminol hemisuccinamide active ester 60:1. Increasing the substitution rate led to a lessening of the dynamic range, shown by an increase in the ratio between unspecific binding (noise) to maximal binding (signal) in an assay. The synthesis of hapten-protein conjugates for covalent coupling to polystyrene balls was undertaken as this formed part of the preparation for the assays described in Part III. The optimal production of gentamicin-bovine **serum albumin** and thyroxine-transferrin conjugates has been described in detail.

L8 ANSWER 21 OF 28 MEDLINE on STN

83108978. PubMed ID: 6185490. Evidence that inhibitors of anion exchange induce a transmembrane conformational change in band 3. Macara I G; Kuo S; Cantley L C. Journal of biological chemistry, **(1983 Feb 10)** 258 (3) 1785-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB The transport inhibitor, eosin 5-**maleimide**, reacts specifically at an external site on the membrane-bound domain of the anion exchange protein, Band 3, in the human erythrocyte membrane. The fluorescence of eosin-labeled resealed ghosts or intact cells was found to be resistant to quenching by CsCl, whereas the fluorescence of labeled inside-out vesicles was quenched by about 27% at saturating CsCl concentrations. Since both Cs+ and eosin **maleimide** were found to be impermeable to the red cell membrane and the vesicles were sealed, these results indicate that after binding of the eosin **maleimide** at the external transport site of Band 3, the inhibitor becomes exposed to ions on the cytoplasmic surface. The lifetime of the bound eosin **maleimide** was determined to be 3 ns both in the absence and presence of CsCl, suggesting that quenching is by a static rather than a dynamic (collisional) mechanism. Intrinsic tryptophan fluorescence of erythrocyte membranes was also investigated using anion transport inhibitors which do not appreciably absorb light at 335 nm. Eosin **maleimide** caused a 25% quenching and 4,4'-dibenzamidodihydrostilbene-2,2'-disulfonate) caused a 7% quenching of tryptophan fluorescence. Covalent labeling of red cells by either eosin **maleimide** or BIDS (4-benzamido-4'-isothiocyanostilbene-2,2'-disulfonate) caused an increase in the susceptibility of membrane tryptophan fluorescence to quenching by CsCl. The quenching constant was similar to that for the quenching of eosin fluorescence and was unperturbed by the presence of 0.5 M KCl. Neither NaCl nor Na citrate produced a large change in the relative magnitude of the tryptophan emission. The tryptophan residues that can be quenched by CsCl appear to be different from those quenched by eosin or BIDS and are possibly located on the cytoplasmic domain of Band 3. The results suggest that a conformational change in the Band 3 protein accompanies the binding of certain anion transport inhibitors to the external transport site of Band 3 and that the inhibitors become exposed on the cytoplasmic side of the red cell membrane.

L8 ANSWER 22 OF 28 MEDLINE on STN

82031835. PubMed ID: 7026686. Novel preparation method of immunogen for hydrophobic hapten, enzyme immunoassay for daunomycin and adriamycin. Fujiwara K; Yasuno M; Kitagawa T. Journal of immunological methods, **(1981)** 45 (2) 195-203. Journal code: 1305440. ISSN: 0022-1759. Pub. country: Netherlands. Language: English.

AB The present study was undertaken to develop a novel method of preparing a hydrophobic hapten as immunogen. The anticancer drug, daunomycin (DM) was used as prototype and coupled to mercaptosuccinylated bovine **serum albumin** (MS, BSA) with N-(gamma-**maleimidobutyryloxy**)succinimide (GMBS). Injection of rabbits with a conjugate (DM-GMBS-MS.BSA) which contained 7.3 DM per BSA molecule produced good levels of anti-DM antibody which was detected by the reaction of diluted antiserum with beta-D-galactosidase-labeled DM. beta-D-galactosidase-labeled DM was used to develop a double antibody enzyme immunoassay for DM and for the DM homologue adriamycin (AM) which reproducibly detected as little as 1 pmole of either drug. A variety of commonly used other anticancer drugs were tested and had little reactivity in this immunoassay. These studies indicate that the anti-DM serum produced is highly specific.

L8 ANSWER 23 OF 28 MEDLINE on STN

82025402. PubMed ID: 6169433. Enzyme immunoassay for pepleomycin, a new bleomycin analog. Fujiwara K; Yasuno M; Kitagawa T. Cancer research, **(1981 Oct)** 41 (10) 4121-6. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB An antibody directed toward pepleomycin, a new antitumor antibiotic related structurally to bleomycin, has been produced in rabbits by immunization with a pepleomycin-protein conjugate which was prepared by a novel procedure of coupling pepleomycin to mercaptosuccinylated bovine **serum albumin** using N-(gamma-**maleimidobutyryloxy**)succinimide as a coupling agent. The antiserum was monospecific to pepleomycin and showed almost no cross-reactivity with a variety of other bleomycin analogs. An enzyme immunoassay for pepleomycin has been developed utilizing this antiserum and beta-D-galactosidase-labeled pepleomycin. The lower limit of detection by this assay, which involves a double antibody technique for the separation of antibody-bound and free antigen, was 50 pg of pepleomycin per tube. Using this assay, drug levels were easily determined in blood and urine of rabbits following administration of pepleomycin in a single dose of 1.2 mg/kg i.v. This assay is also suitable for measuring pepleomycin in the presence of other drugs since the assay is not significantly affected by any other antineoplastic agents tested. Since pepleomycin is now undergoing clinical trial, the enzyme immunoassay of the drug will be a valuable tool in clinical **pharmacological** studies.

L8 ANSWER 24 OF 28 MEDLINE on STN

80226859. PubMed ID: 6248464. Initial kinetics of lysosomal enzyme secretion and superoxide anion generation by human polymorphonuclear leukocytes. Smolen J E; Korchak H M; Weissmann G. Inflammation, **(1980 Jun)** 4 (2) 145-63. Journal code: 7600105. ISSN: 0360-3997. Pub. country: United States. Language: English.

AB Human polymorphonuclear leukocytes (PMN) exposed to particulate and soluble stimuli secrete lysosomal enzymes. These stimuli cause prompt (less than 10 sec) changes in membrane potential followed 30--45 sec later by superoxide anion (O-2.) production. We describe a new technique utilizing flow dialysis apparatus which monitors the first stages of lysosomal enzyme release with a resolution of approximately 6 sec. Secretion of beta-glucuronidase from cytochalasin B-treated PMN could be detected 19+/-5 sec after exposure to the chemotactic peptide N-formylmethionylleucylphenylalanine (FMLP). The "lag" times for release of this enzyme were different for other stimuli: 35+/-8 sec (BSA/anti-BSA immune complex); 48+/-8 sec (serum-treated zymosan, "STZ"); 60+/-25 sec (calcium ionophore A23187). The lag times for lysozyme release were less dependent upon the stimulus presented (28+/-16 sec for FMLP, 28+/-8 sec fo BSA/anti-BSA, 32+/-10 sec for STZ, and 38+/-8 seconds for Con A); only A23187 had a long lag period: 74+/-27 sec. Lag periods for the onset of O-2. production (measured by the same mathematical criteria) were comparable to those for beta-glucuronidase release: 21+/-4 sec for FMLP, 43+/-14 sec for BSA/anti-BSA, 62+/-7 sec for Con A, and 50+/-13 sec for A23187. Changes in FMLP dose up to 100-fold affected the magnitudes of O-2. generation and beta-glucuronidase release, but did not alter the time required for the onset of these processes. A variety of agents, such as corticosteroids, colchicine, 2-deoxyglucose, and N-ethyl **maleimide**, also affected the magnitudes of the responses, but not the lag periods when FMLP was used as the stimulus. When BSA/anti-BSA immune complex was used as the stimulus, 2-deoxyglucose and N-ethyl **maleimide** increased the lag period for superoxide anion generation, but not for lysosomal enzyme release. This new flow dialysis technique has permitted us to demonstrate the O-2. production and lysosomal enzyme secretion are concurrent but dissociable processes which are subsequent to earlier responses of the granulocyte-to-ligand-receptor interactions as reflected by changes in membrane potential.

L8 ANSWER 25 OF 28 MEDLINE on STN

78192381. PubMed ID: 77827. Studies on D-penicillamine (1): Inhibitory effect of D-penicillamine on the heat-Cu++ induced denaturation of human gamma-globulin (author's transl). Otomo S; Takahashi K; Sasajima M; Tanaka I. Nippon yakurigaku zasshi. Japanese journal of pharmacology, **(1978 Mar)** 74 (2) 193-205. Journal code: 0420550. ISSN: 0015-5691. Pub. country: Japan. Language: Japanese.

AB The inhibitory effect of D-penicillamine on the denaturation of human gamma-globulin induced by heat and Cu++ was compared with the action of other agents such as antirheumatic drugs, anti-inflammatory drugs, SH reagents, SH inhibitors and chelating reagents. The denaturation of human gamma globulin was induced by Cu++ at 10 micrometer ("Cu++ induced denaturation") and was further increased by heating at 63 degrees C for 3 hr in the presence of Cu++ ("total denaturation"). Thus the value obtained by subtracting "Cu++ induced denaturation" from "total one" was designated as "thermal denaturation". D-Penicillamine enhanced "thermal denaturation" at a low concentration but inhibited it with increasing the concentration as well as L-cysteine. SH reagents such as thiomalic acid, 6-mercaptopurine inhibited "total" and "thermal" denaturation. SH inhibitors and protein binding reagents such as N-ethylmaleimide, trinitrobenzenesulfonic acid inhibited the "total" and "thermal"

denaturation of the protein. Chelating reagents such as ethylenediamine tetraacetic acid, 8-hydroxyquinoline inhibited "total", "Cu++ induced" and "thermal" denaturation of the protein. Au+ inhibited "total denaturation", but not "Cu++ induced denaturation". On the other hand, Au+++ denaturated the protein considerably with or without heating, in the absence of Cu++ but dithiothreitol did so only with heating in the same condition. The anti-inflammatory drugs used herein had no effect on the protein denaturation. D-Penicillamine apparently prevents the denaturation of human gamma-globulin by the chelate formation with Cu++ and the binding to free protein SH, initiator for sulfhydryldisulfide interchange reaction.

L8 ANSWER 26 OF 28 MEDLINE on STN
78162537. PubMed ID: 347850. [Immobilization of proteins on macroporous glasses involving maleinimide as the anchoring group]. Immobilisierung von Proteinen an makroporosen Glasern unter Beteiligung von Maleinimid als Ankergruppe. Fischer J; Heyer W; Janowski F; Wolf F; Schellenberger A. Acta biologica et medica Germanica, **(1977)** 36 (7-8) 999-1005. Journal code: 0370276. ISSN: 0001-5318. Pub. country: GERMANY, EAST: German Democratic Republic. Language: German.

AB Macroporous glasses with pore sizes from 400-1000 A appropriate for protein binding were produced and characterized by a thermal demixing procedure and alkaline after treatment. To achieve a covalent binding capacity relative to proteins, the gamma-aminopropyl derivative was allowed to react with 4-maleinimido benzoylic chloride to give preparations containing, in addition to maleinimide residues, acid chloride structures for the protein binding. A preparation of 400 A pore size was tested for its protein binding capacity relative to bovine **serum albumin** and trypsin. Furthermore, the capacity of binding glucoamylase from Endomycopsis bispora in active form was studied.

L8 ANSWER 27 OF 28 MEDLINE on STN
75187762. PubMed ID: 166896. Degradation of insulin by isolated rat liver cells. Le Cam A; Freychet P; Lenoir P. Diabetes, **(1975 Jun)** 24 (6) 566-73. Journal code: 0372763. ISSN: 0012-1797. Pub. country: United States. Language: English.

AB The degradation of insulin by isolated rat liver cells has been studied. The phenomenon is time- and temperature-dependent. After sixty minutes' exposure to 1.5 times 10-6 cells/ml, about 50 per cent, 15 per cent, and less than 5 per cent of insulin at 1.5 muM. are degraded at 37 degrees C., 20 degrees, and 0 degrees C., respectively. The methods used to measure the hormone degradation effect the apparent Vmax. Higher values of Vmax are found when radioimmunoassay rather than precipitation by trichloracetic acid and absorption to talc is used. However, the apparent Km. (0.27 muM) is virtually the same with any of methods used. N-ethyl-**maleimide** and Trasylol are potent inhibitors, whereas GSH increases the hormone degradation. Proinsulin acts as competitive inhibitor (apparent Ki equals 0.35 muM.). Gel filtration patterns of incubation supernates suggest that several enzymatic systems may be operative in the degradation of insulin by the liver cells. Glutathione-insulin-transhydrogenase is suggested by the appearance of a component that has the same elution volume as the A chain, but the inhibitory effects of trasylol on insulin degradation, as well as qualitative and quantitative similarities with insulin proteases, suggest that a proteolytic similiarities with insulin proteases, suggest that a proteolytic mechanism is involved. The insulin-degrading system in isolated liver cells closely resembles that observed in purified liver plasma membranes and in the isolated perfused liver. Such similarities stress the possible significance of the degradation process in the regulation of insulin action. These studies are also important for the quantitative analysis of insulin interaction with its specific receptors in isolated liver cells.

L8 ANSWER 28 OF 28 MEDLINE on STN
72013885. PubMed ID: 4999810. [In vitro study of the transport of N-Ethyl-**maleimide** by serum proteins]. Etude in vitro du transport du N-ethyl-**maleimide** par les proteines seriques. Cohen Y; Jullien N. Comptes rendus hebdomadaires des seances de l'Academie des sciences. Serie D: Sciences naturelles, **(1971 Jul 19)** 273 (3) 429-31. Journal code: 7501107. ISSN: 0567-655X. Pub. country: France. Language: French.

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L1 68146 S (SERUM ALBUMIN)
L2 74 S L1 AND (CYS-34 OR CYSTEINE 34)

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L3           39 S L2 AND PY<2000
L4        15041 S L1 AND PHARMACOLOG?
L5         1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6            2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
L7           47 S L4 AND (MALEIMID?)
L8           28 S L7 AND PY<2000

=> file uspatful
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                   ENTRY    SESSION
FULL ESTIMATED COST                               27.62      27.83

FILE 'USPATFULL' ENTERED AT 20:20:48 ON 26 NOV 2005
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FILE COVERS 1971 TO PATENT PUBLICATION DATE: 24 Nov 2005 (20051124/PD)
FILE LAST UPDATED: 24 Nov 2005 (20051124/ED)
HIGHEST GRANTED PATENT NUMBER: US6968571
HIGHEST APPLICATION PUBLICATION NUMBER: US2005262612
CA INDEXING IS CURRENT THROUGH 24 Nov 2005 (20051124/UPCA)
ISSUE CLASS FIELDS (/INCL) CURRENT THROUGH: 24 Nov 2005 (20051124/PD)
REVISED CLASS FIELDS (/NCL) LAST RELOADED: Oct 2005
USPTO MANUAL OF CLASSIFICATIONS THESAURUS ISSUE DATE: Oct 2005

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>>>  USPATFULL.  A USPATFULL record contains not only the original     <<<
>>>  published document but also a list of any subsequent              <<<
>>>  publications.  The publication number, patent kind code, and      <<<
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>>>  /PK, etc.                                                         <<<

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>>>  Use USPATALL when searching terms such as patent assignees,       <<<
>>>  classifications, or claims, that may potentially change from      <<<
>>>  the earliest to the latest publication.                           <<<

This file contains CAS Registry Numbers for easy and accurate
substance identification.

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         83951 ALBUMIN
L9       59637 (SERUM ALBUMIN)
                (SERUM(W)ALBUMIN)

=> d his

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L8          28 S L7 AND PY<2000

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=> d l11,cbib,ab,clm,1-13

L11 ANSWER 1 OF 13 USPATFULL on STN
2003:314573 Oligonucleotides conjugated to protein-binding drugs.
Manoharan, Muthiah, Carlsbad, CA, United States
ISIS Pharmaceuticals, Inc., Carlsbad, CA, United States (U.S. corporation)
US 6656730 B1 20031202
APPLICATION: US 1999-334130 19990615 (9)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Ligand-conjugated oligomeric compounds are described wherein ligands are conjugated to one or more sites on an oligomeric compound including the 2'-, 3'-, 5'-, nucleobase and internucleotide linkage sites. The ligand can be attached via an optional linking group. Ligands are selected for conjugation that bind to one or more cellular, serum or vascular proteins imparting enhanced pharmacokinetic properties to the resulting ligand-conjugated oligomeric compounds. Also provided are methods for increasing the concentration of an oligonucleotide in serum and methods for increasing the capacity of serum for an oligonucleotide. Further, methods for increasing the binding of an oligonucleotide to a portion of the vascular system is described. Also provided are methods for promoting cellular uptake of an oligonucleotide in cells.
CLM What is claimed is:
1. An oligonucleotide covalently attached to an arylpropionic acid that interacts with human **serum albumin**, wherein the arylpropionic acid is selected from the group consisting of ibuprofen, suprofen, ketoprofen, (S)-(+)-pranoprofen, and carprofen.

2. The oligonucleotide of claim 1 wherein said arylpropionic acid binds to said human **serum albumin**.

3. The oligonucleotide of claim 1 wherein said oligonucleotide comprises a plurality of nucleosides connected by covalent internucleoside linkages.

4. The oligonucleotide of claim 3 wherein said linkages are phosphodiester linkages.

5. The oligonucleotide of claim 3 wherein said linkages are phosphorothioate linkages.

6. The oligonucleotide of claim 3 wherein said linkages are non-phosphorus-containing linkages.

7. The oligonucleotide of claim 3 wherein at least one of said nucleosides bears a 2'-substituent group.

8. The oligonucleotide of claim 7 wherein said 2'-substituent group is O-alkylalkoxy.

9. The oligonucleotide of claim 8 wherein said 2'-sustituent group is methoxyethoxy.

10. A method of increasing the concentration of an oligonucleotide in human serum comprising the steps of: (a) selecting an arylpropionic acid that is known to bind to human **serum albumin**; (b) covalently-attaching said arylpropionic acid to said oligonucleotide to form a conjugated oligonucleotide; and (c) adding said conjugated oligonucleotide to said human serum, wherein the concentration of said oligonucleotide in human serum is increased; and wherein the arylpropionic acid is selected from the group consisting of ibuprofen, suprofen, keroprofen, (S)-(+)-pranoprofen, and carprofen.

11. The method claim 10 wherein said arylpropionic acid is ibuprofen.

12. A method of increasing the capacity of human serum for an oligonucleotide comprising the steps of: (a) selecting an arylpropionic acid that is known to bind to human **serum albumin**; (b) covalently attaching said arylpropionic acid to said oligonucleotide to form a conjugated oligonucleotide; and (c) adding said conjugated oligonucleotide to said human serum, wherein the capacity of human serum is increased for said oligonucleotide; and wherein the arylpropionic acid is selected from the group consisting of ibuprofen, suprofen,

ketoprofen, (S)-(+)-pranoprofen, and carprofen.

13. The method of claim 11 wherein said human **serum albumin** has a binding site for said oligonucleotide and a binding site for said arylpropionic acid; wherein said binding site for said oligonucleotide is distinct from said binding site for said arylpropionic acid.

L11 ANSWER 2 OF 13 USPATFULL on STN
2003:53885 Precerebellin-like protein.
Young, Paul, Gaithersburg, MD, United States
Greene, John M., Gaithersburg, MD, United States
Ferrie, Ann M., Tewksbury, MA, United States
Ruben, Steven M., Olney, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Hu, Jing-Shan, Sunnyvale, CA, United States
Olsen, Henrik S., Gaithersburg, MD, United States
Ebner, Reinhard, Gaithersburg, MD, United States
Brewer, Laurie A., St. Paul, MN, United States
Moore, Paul A., Germantown, MD, United States
Shi, Yanggu, Gaithersburg, MD, United States
Florence, Charles, Rockville, MD, United States
Florence, Kimberly, Rockville, MD, United States
Lafleur, David W., Washington, DC, United States
Ni, Jian, Rockville, MD, United States
Fan, Ping, Gaithersburg, MD, United States
Wei, Ying-Fei, Berkeley, CA, United States
Fischer, Carrie L., Burke, VA, United States
Soppet, Daniel R., Centreville, VA, United States
Li, Yi, Sunnyvale, CA, United States
Zeng, Zhizhen, Gaithersburg, MD, United States
Kyaw, Hla, Frederick, MD, United States
Yu, Guo-Liang, Berkeley, CA, United States
Feng, Ping, Gaithersburg, MD, United States
Dillon, Patrick J., Carlsbad, CA, United States
Endress, Gregory A., Potomac, MD, United States
Carter, Kenneth C., North Potomac, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6525174 B1 20030225
APPLICATION: US 1998-205258 19981204 (9)
PRIORITY: US 1997-70923P 19971218 (60)
US 1998-94657P 19980730 (60)
US 1997-48885P 19970606 (60)
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US 1997-70923P 19971218 (60)
US 1998-92921 19980715 (09)
US 1998-94657P 19980730 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CLM What is claimed is:

1. An isolated protein comprising amino acid residues 33 to 205 of SEQ ID NO:463.

2. The isolated protein of claim 1 which comprises amino acid residues 2 to 205 of SEQ ID NO:463.

3. The isolated protein of claim 1 which comprises amino acid residues 1 to 205 of SEQ ID NO:463.

4. The protein of claim 1 which comprises a heterologous polypeptide sequence.

5. A composition comprising the protein of claim 1 and a pharmaceutically acceptable carrier.

6. An isolated protein produced by the method comprising: (a) expressing the protein of claim 1 by a cell; and (b) recovering said protein.

7. An isolated protein comprising a polypeptide sequence which is at least 95% identical to amino acid residues 33 to 205 of SEQ ID NO:463.

8. The isolated protein of claim 7 wherein said polypeptide sequence is at least 95% identical to amino acid residues 1 to 205 of SEQ ID NO:463.

9. The protein of claim 7 which comprises a heterologous polypeptide sequence.

10. A composition comprising the protein of claim 7 and a pharmaceutically acceptable carrier.

11. An isolated protein produced by the method comprising: (a) expressing the protein of claim 7 by a cell; and (b) recovering said protein.

12. An isolated protein comprising at least 50 contiguous amino acid residues of amino acid residues 33 to 205 of SEQ ID NO:463.

13. The protein of claim 12 which comprises a heterologous polypeptide sequence.

14. A composition comprising the protein of claim 12 and a pharmaceutically acceptable carrier.

15. An isolated protein produced by the method comprising: (a) expressing the protein of claim 12 by a cell; and (b) recovering said protein.

16. An isolated protein comprising at least 50 contiguous amino acid residues of amino acid residues 1 to 205 of SEQ ID NO:463.

17. The protein of claim 16 which comprises a heterologous polypeptide sequence.

18. A composition comprising the protein of claim 16 and a pharmaceutically acceptable carrier.

19. An isolated protein produced by the method comprising: (a) expressing the protein of claim 16 by a cell; and (b) recovering said protein.

L11 ANSWER 3 OF 13 USPATFULL on STN
2002:175279 186 human secreted proteins.
Ruben, Steven M., Olney, MD, United States
Rosen, Craig A., Laytonsville, MD, United States
Fischer, Carrie L., Burke, VA, United States
Soppet, Daniel P., Centreville, VA, United States
Carter, Kenneth C., North Potomac, MD, United States
Bednarik, Daniel R., Columbia, MD, United States
Endress, Gregory A., Potomac, MD, United States
Yu, Guo-Liang, Berkeley, CA, United States
Ni, Jian, Rockville, MD, United States
Feng, Ping, Gaithersburg, MD, United States
Young, Paul E., Gaithersburg, MD, United States
Greene, John M., Gaithersburg, MD, United States
Ferrie, Ann M., Tewksbury, MA, United States
Duan, Roxanne, Bethesda, MD, United States
Hu, Jing-Shan, Sunnyvale, CA, United States
Florence, Kimberly A., Rockville, MD, United States
Olsen, Henrik S., Gaithersburg, MD, United States
Ebner, Reinhard, Gaithersburg, MD, United States
Brewer, Laurie A., St. Paul, MN, United States
Moore, Paul A., Germantown, MD, United States
Shi, Yanggu, Gaithersburg, MD, United States
Lafleur, David W., Washington, DC, United States
Li, Yi, Sunnyvale, CA, United States
Zeng, Zhizhen, Lansdale, PA, United States
Kyaw, Hla, Frederick, MD, United States
Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)
US 6420526 B1 20020716
APPLICATION: US 1998-149476 19980908 (9)
PRIORITY: US 1997-40162P 19970307 (60)
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US 1997-55724P 19970818 (60)
US 1997-40161P 19970307 (60)
DOCUMENT TYPE: Utility; GRANTED.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to novel human secreted proteins and isolated nucleic acids containing the coding regions of the genes encoding such proteins. Also provided are vectors, host cells, antibodies, and recombinant methods for producing human secreted proteins. The invention further relates to diagnostic and therapeutic methods useful for diagnosing and treating disorders related to these novel human secreted proteins.

CLM What is claimed is:

1. An isolated protein comprising amino acid residues 27 to 234 of SEQ ID NO:478.

2. The isolated protein of claim 1 which comprises amino acid residues 2 to 234 of SEQ ID NO:478.

3. The isolated protein of claim 1 which comprises amino acid residues 1 to 234 of SEQ ID NO:478.

4. The protein of claim 1 which comprises a heterologous polypeptide sequence.

5. A composition comprising the protein of claim 1 and a pharmaceutically acceptable carrier.

6. An isolated protein produced by the method comprising: (a) expressing the protein of claim 1 by a cell; and (b) recovering said protein.

7. An isolated protein comprising the amino acid sequence of the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

8. The isolated protein of claim 7 which comprises the amino acid sequence of the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903, excepting the N-terminal methionine.

9. The isolated protein of claim 7 which comprises the amino acid sequence of the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

10. The protein of claim 7 which comprises a heterologous polypeptide sequence.

11. A composition comprising the protein of claim 7 and a pharmaceutically acceptable carrier.

12. An isolated protein produced by the method comprising: (a) expressing the protein of claim 7 by a cell; and (b) recovering said protein.

13. An isolated protein comprising a polypeptide sequence which is at least 90% identical to amino acid residues 27 to 234 of SEQ ID NO:478.

14. The isolated protein of claim 13 wherein said polypeptide sequence is at least 90% identical to amino acid residues 1 to 234 of SEQ ID NO:478.

15. The isolated protein of claim 13 wherein said polypeptide sequence is at least 95% identical to amino acid residues 27 to 234 of SEQ ID NO:478.

16. The isolated protein of claim 13 wherein said polypeptide sequence is at least 95% identical to amino acid residues 1 to 234 of SEQ ID NO:478.

17. The protein of claim 13 which comprises a heterologous polypeptide sequence.

18. A composition comprising the protein of claim 13 and a pharmaceutically acceptable carrier.

19. An isolated protein produced by the method comprising: (a) expressing the protein of claim 13 by a cell; and (b) recovering said protein.

20. An isolated protein comprising a polypeptide sequence which is at least 90% identical to the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

21. The isolated protein of claim 20 wherein saod polypeptide sequence is at least 95% identical to the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

22. The protein of claim 20 which comprises a heterologous polypeptide sequence.

23. A composition comprising the protein of claim 20 and a pharmaceutically acceptable carrier.

24. An isolated protein produced by the method comprising: (a) expressing the protein of claim 20 by a cell; and (b) recovering said protein.

25. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 27 to 234 of SEQ ID NO:478.

26. The isolated protein of claim 25 which comprises at least 50 contiguous amino acid residues of amino acid residues 27 to 234 of SEQ ID NO:478.

27. The protein of claim 25 which comprises a heterologous polypeptide sequence.

28. A composition comprising the protein of claim 25 and a pharmaceutically acceptable carrier.

29. An isolated protein produced by the method comprising: (a) expressing the protein of claim 25 by a cell; and (b) recovering said protein.

30. An isolated protein comprising at least 30 contiguous amino acid residues of the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

31. The isolated protein of claim 30 which comprises at least 50 contiguous amino acid residues of the secreted portion of the polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

32. The protein of claim 30 which comprises a heterologous polypeptide sequence.

33. A composition comprising the protein of claim 30 and pharmaceutically acceptable carrier.

34. An isolated protein produced by the method comprising: (a) expressing the protein of claim 30 by a cell; and (b) recovering said protein.

35. An isolated protein comprising at least 30 contiguous amino acid residues of amino acid residues 1 to 234 of SEQ ID NO:478.

36. The isolated protein of claim 35 which comprises at least 50 contiguous amino acid residues of amino acid residues 1 to 234 of SEQ ID NO:478.

37. The protein of claim 35 which comprises a heterologous polypeptide sequence.

38. A composition comprising the protein of claim 35 and a pharmaceutically acceptable carrier.

39. An isolated protein produced by the method comprising: (a) expressing the protein of claim 35 by a cell; and (b) recovering said protein.

40. An isolated protein comprising at least 30 contiguous amino acid residues of the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

41. The isolated protein of claim 40 which comprises at least 50 contiguous amino acid residues of the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

42. The protein of claim 40 which comprises a heterologous polypeptide sequence.

43. A composition comprising the protein of claim 40 and pharmaceutically acceptable carrier.

44. An isolated protein produced by the method comprising: (a) expressing the protein of claim 40 by a cell; and (b) recovering said protein.

45. An isolated protein comprising a polypeptide sequence which is at least 90% identical to the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

46. The isolated protein of claim 45, wherein said polypeptide sequence is at least 95% identical to the complete polypeptide encoded by the HNFIP24 cDNA contained in ATCC Deposit No. 97903.

47. The protein of claim 45 which comprises a heterologous polypeptide sequence.

48. The protein of claim 47, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

49. A composition comprising the protein of claim 45 and a pharmaceutically acceptable carrier.

50. An isolated protein produced by a method comprising: (a) expressing the protein of claim 45 by a cell; and (b) recovering said protein.

51. The protein of claim 4, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

52. The protein of claim 10, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

53. The protein of claim 17, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

54. The protein of claim 22, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

55. The protein of claim 27, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

56. The protein of claim 32, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

57. The protein of claim 37, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

58. The protein of claim 42, wherein said heterologous polypeptide sequence is the Fc domain of immunoglobulin.

L11 ANSWER 4 OF 13 USPATFULL on STN

2002:81240 Polynucleotides encoding chemokine α-6.

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Ruben, Steven M., Olney, MD, United States
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Human Genome Sciences, Inc., Rockville, MD, United States (U.S. corporation)

US 6372456 B1 20020416
APPLICATION: US 1998-177304 19981023 (9)
PRIORITY: US 1997-63387P 19971024 (60)
US 1998-79245P 19980325 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to a novel CK(α-6 protein which is a member of the alpha chemokine family. In particular, isolated nucleic acid molecules are provided encoding the human CKα-6 protein. CKα-6 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of CKα-6 activity. Also provided are diagnostic methods for detecting CNS and immune system-related disorders and therapeutic methods for treating CNS and immune system-related disorders.

CLM What is claimed is:

1. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues +1 to +84 of SEQ ID NO:2; (b) a nucleic acid sequence encoding amino acid residues +2 to +84 of SEQ ID NO:2; (c) a nucleic acid sequence encoding amino acid residues +17 to +84 of SEQ ID NO:2; (d) a nucleic acid sequence encoding amino acid residues +18 to +84 of SEQ ID NO:2; (e) a nucleic acid sequence encoding amino acid residues +19 to +84 of SEQ ID NO:2; (f) a nucleic acid sequence encoding amino acid residues +20 to +84 of SEQ ID NO:2; (g) a nucleic acid sequence encoding amino acid residues +21 to +84 of SEQ ID NO:2; and (h) a nucleic acid sequence encoding amino acid residues +22 to +84 of SEQ ID NO:2.

2. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (a).

3. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (b).

4. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (c).

5. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (d).

6. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (e).

7. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (f).

8. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (g).

9. The isolated polynucleotide of claim 1 which comprises nucleic acid sequence (h).

10. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 1.

11. The isolated polynucleotide of claim 1 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

12. The isolated polynucleotide of claim 11 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

13. The isolated polynucleotide of claim 12 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

14. A recombinant vector comprising the isolated polynucleotide of claim 1.

15. The recombinant vector of claim 14 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

16. A recombinant host cell comprising the isolated polynucleotide of claim 1.

17. The recombinant host cell of claim 16 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

18. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 1; and (b) recovering the protein from the cell culture.

19. A composition comprising the isolated polynucleotide of claim 1 and a pharmaceutically acceptable carrier.

20. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide encoded by the cDNA in ATCC Deposit No. 209643; (b) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide, excluding the N-terminal methionine residue, encoded by the cDNA in ATCC Deposit No. 209643; and (c) a nucleic acid sequence encoding the amino

acid sequence of the mature polypeptide encoded by the cDNA in ATCC Deposit No. 209643.

21. The isolated polynucleotide of claim 20 which comprises nucleic acid sequence (a).

22. The isolated polynucleotide of claim 20 which comprises nucleic acid sequence (b).

23. The isolated polynucleotide of claim 20 which comprises nucleic acid sequence (c).

24. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 20.

25. The isolated polynucleotide of claim 20 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

26. The isolated polynucleotide of claim 25 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

27. The isolated polynucleotide of claim 26 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

28. A recombinant vector comprising the isolated polynucleotide of claim 20.

29. The recombinant vector of claim 28 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

30. A recombinant host cell comprising the isolated polynucleotide of claim 20.

31. The recombinant host cell of claim 30 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

32. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 20; and (b) recovering the protein from the cell culture.

33. A composition comprising the isolated polynucleotide of claim 20 and a pharmaceutically acceptable carrier.

34. An isolated polynucleotide comprising a first nucleic acid sequence 90% or more identical to a second nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues +1 to +84 of SEQ ID NO:2; (b) a nucleic acid sequence encoding amino acid residues +2 to +84 of SEQ ID NO:2; (c) a nucleic acid sequence encoding amino acid residues +17 to +84 of SEQ ID NO:2; (d) a nucleic acid sequence encoding amino acid residues +18 to +84 of SEQ ID NO:2; (e) a nucleic acid sequence encoding amino acid residues +19 to +84 of SEQ ID NO:2; (f) a nucleic acid sequence encoding amino acid residues +20 to +84 of SEQ ID NO:2; (g) a nucleic acid sequence encoding amino acid residues +21 to +84 of SEQ ID NO:2; and (h) a nucleic acid sequence encoding amino acid residues +22 to +84 of SEQ ID NO:2; wherein percent identity is calculated using FASTDB with the parameters set such that percentage of identity is calculated over the full length of the reference nucleic acid sequence and that gaps in homology of up to 5% of the total number of nucleic acids in the reference nucleic acid sequence are allowed.

35. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (a).

36. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (b).

37. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (c).

38. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (d).

39. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (e).

40. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (f).

41. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (g).

42. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (h).

43. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (a).

44. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (b).

45. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (c).

46. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (d).

47. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (e).

48. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (f).

49. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (g).

50. The isolated polynucleotide of claim 34 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (h).

51. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 34.

52. The isolated polynucleotide of claim 34 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

53. The isolated polynucleotide of claim 52 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

54. The isolated polynucleotide of claim 53 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

55. A recombinant vector comprising the isolated polynucleotide of claim 34.

56. The recombinant vector of claim 55 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

57. A recombinant host cell comprising the isolated polynucleotide of claim 34.

58. The recombinant host cell of claim 57 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

59. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 34; and (b) recovering the protein from the cell culture.

60. A composition comprising the isolated polynucleotide of claim 34 and a pharmaceutically acceptable carrier.

61. An isolated polynucleotide comprising a first nucleic acid sequence 90% or more identical to a second nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide encoded by the cDNA in ATCC Deposit No. 209643; (b) a nucleic acid sequence encoding the amino acid sequence of the full-length polypeptide, excluding the N-terminal methionine residue, encoded by the cDNA in ATCC Deposit No. 209643; and (c) a nucleic acid sequence encoding the amino acid sequence of the mature polypeptide encoded by the cDNA in ATCC Deposit No. 209643; wherein percent identity is calculated using FASTDB with the parameters set such that percentage of identity is calculated over the full length of the reference nucleic acid sequence and that gaps in homology of up

to 5% of the total number of nucleic acids in the reference nucleic acid sequence are allowed.

62. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (a).

63. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (b).

64. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 90% identical to said second nucleic acid sequence (c).

65. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (a).

66. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (b).

67. The isolated polynucleotide of claim 61 wherein said first nucleic acid sequence is 95% identical to said second nucleic acid sequence (c).

68. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 61.

69. The isolated polynucleotide of claim 61 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

70. The isolated polynucleotide of claim 69 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

71. The isolated polynucleotide of claim 70 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

72. A recombinant vector comprising the isolated polynucleotide of claim 61.

73. The recombinant vector of claim 72 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

74. A recombinant host cell comprising the isolated polynucleotide of claim 61.

75. The recombinant host cell of claim 74 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

76. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 61; and (b) recovering the protein from the cell culture.

77. A composition comprising the isolated polynucleotide of claim 61 and a pharmaceutically acceptable carrier.

78. An isolated polynucleotide comprising a nucleic acid sequence which hybridizes to the complement of the nucleic acid sequence set forth in SEQ ID NO:1 wherein said hybridization occurs under conditions comprising hybridization in a buffer consisting essentially of 50% formamide, 5×SSC, 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 ug/ml denatured, sheared salmon sperm DNA at 42° C. and wash in a solution consisting of 0.1×SSC at 65° C.

79. The isolated polynucleotide of claim 78 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

80. The isolated polynucleotide of claim 79 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

81. The isolated polynucleotide of claim 80 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

82. A recombinant vector comprising the isolated polynucleotide of claim 78.

83. The recombinant vector of claim 82 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

84. A recombinant host cell comprising the isolated polynucleotide of claim 78.

85. The recombinant host cell of claim 84 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

86. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 78; and (b) recovering the protein from the cell culture.

87. A composition comprising the isolated polynucleotide of claim 78 and a pharmaceutically acceptable carrier.

88. An isolated polynucleotide comprising a nucleic acid sequence which hybridizes to the cDNA in ATCC Deposit No. 209643 wherein said hybridization occurs under conditions comprising hybridization in a buffer consisting essentially of 50% formamide, 5×SSC, 50 mM sodium phosphate (pH 7.6), 5×Denhardt's solution, 10% dextran sulfate, and 20 ug/ml denatured, sheared salmon sperm DNA at 42° C. and wash in a solution consisting of 0.1×SSC at 65°.

89. The isolated polynucleotide of claim 88 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

90. The isolated polynucleotide of claim 89 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

91. The isolated polynucleotide of claim 90 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

92. A recombinant vector comprising the isolated polynucleotide of claim 88.

93. The recombinant vector of claim 92 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

94. A recombinant host cell comprising the isolated polynucleotide of claim 88.

95. The recombinant host cell of claim 94 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

96. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 88; and (b) recovering the protein from the cell culture.

97. A composition comprising the isolated polynucleotide of claim 88 and a pharmaceutically acceptable carrier.

98. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues +26 to +34 of SEQ ID NO:2; (b) a nucleic acid sequence encoding amino acid residues +36 to +45 of SEQ ID NO:2; (c) a nucleic acid sequence encoding amino acid residues +58 to +66 of SEQ ID NO:2; and (d) a nucleic acid sequence encoding amino acid residues +77 to +84 of SEQ ID NO:2.

99. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (a).

100. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (b).

101. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (c).

102. The isolated polynucleotide of claim 98 which comprises nucleic acid sequence (d).

103. An isolated nucleic acid molecule complementary to the isolated polynucleotide of claim 98.

104. The isolated polynucleotide of claim 98 wherein said nucleic acid

sequence further comprises a heterologous nucleic acid sequence.

105. The isolated polynucleotide of claim 104 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

106. The isolated polynucleotide of claim 105 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

107. A recombinant vector comprising the isolated polynucleotide of claim 98.

108. The recombinant vector of claim 107 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

109. A recombinant host cell comprising the isolated polynucleotide of claim 98.

110. The recombinant host cell of claim 109 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

111. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 98; and (b) recovering the protein from the cell culture.

112. A composition comprising the isolated polynucleotide of claim 98 and a pharmaceutically acceptable carrier.

113. An isolated polynucleotide comprising a nucleic acid sequence which encodes at least 30 contiguous amino acid residues of SEQ ID NO:2.

114. The isolated polynucleotide of claim 113 which comprises a nucleic acid sequence which encodes at least 50 contiguous amino acid residues of SEQ ID NO:2.

115. The isolated polynucleotide of claim 113 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

116. The isolated polynucleotide of claim 115 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

117. The isolated polynucleotide of claim 116 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

118. A recombinant vector comprising the isolated polynucleotide of claim 113.

119. The recombinant vector of claim 118 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

120. A recombinant host cell comprising the isolated polynucleotide of claim 113.

121. The recombinant host cell of claim 120 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

122. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 113; and (b) recovering the protein from the cell culture.

123. A composition comprising the isolated polynucleotide of claim 113 and a pharmaceutically acceptable carrier.

124. An isolated polynucleotide comprising a nucleic acid sequence which encodes at least 30 contiguous amino acid residues encoded by the cDNA in ATCC Deposit No. 209643.

125. The isolated polynucleotide of claim 124 which comprises a nucleic acid sequence which encodes at least 50 contiguous amino acid residues encoded by the cDNA in ATCC Deposit No. 209643.

126. The isolated polynucleotide of claim 124 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

127. The isolated polynucleotide of claim 126 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

128. The isolated polynucleotide of claim 127 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

129. A recombinant vector comprising the isolated polynucleotide of claim 124.

130. The recombinant vector of claim 129 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

131. A recombinant host cell comprising the isolated polynucleotide of claim 124.

132. The recombinant host cell of claim 131 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

133. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 124; and (b) recovering the protein from the cell culture.

134. A composition comprising the isolated polynucleotide of claim 124 and a pharmaceutically acceptable carrier.

135. An isolated polynucleotide comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence encoding amino acid residues n to 84 of SEQ ID NO:2, where n is an integer in the range of 17 to 22; (b) a nucleic acid sequence encoding amino acid residues 17 to m of SEQ ID NO:2, where m is an integer in the range of 68 to 84; and (c) a nucleic acid sequence encoding amino acid residues n to m of SEQ ID NO:2, where n is an integer in the range of 17 to 22 and m is an integer in the range of 68 to 84; wherein said nucleic acid sequence is not Genbank Accession No. AA410918.

136. The isolated polynucleotide of claim 135 which comprises nucleic acid sequence (a).

137. The isolated polynucleotide of claim 135 which comprises nucleic acid sequence (b).

138. The isolated polynucleotide of claim 135 which comprises nucleic acid sequence (c).

139. The isolated polynucleotide of claim 135 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

140. The isolated polynucleotide of claim 139 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

141. The isolated polynucleotide of claim 140 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

142. A recombinant vector comprising the isolated polynucleotide of claim 135.

143. The recombinant vector of claim 142 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

144. A recombinant host cell comprising the isolated polynucleotide of claim 135.

145. The recombinant host cell of claim 144 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

146. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 135; and (b) recovering the protein from the cell culture.

147. A composition comprising the isolated polynucleotide of claim 135 and a pharmaceutically acceptable carrier.

148. An isolated polynucleotide comprising a nucleic acid sequence

selected from the group consisting of: (a) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment inhibits endothelial cell function; (b) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment inhibits inflammation; (c) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment is angiostatic; and (d) a nucleic acid sequence encoding a fragment of SEQ ID NO:2, wherein said fragment binds an antibody specific for the polypeptide encoded by SEQ ID NO:2.

149. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (a).

150. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (b).

151. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (c).

152. The isolated polynucleotide of claim 148 which comprises nucleic acid sequence (d).

153. The isolated polynucleotide of claim 148 wherein said nucleic acid sequence further comprises a heterologous nucleic acid sequence.

154. The isolated polynucleotide of claim 153 wherein said heterologous nucleic acid sequence encodes a heterologous polypeptide.

155. The isolated polynucleotide of claim 154 wherein said heterologous polypeptide is the Fc domain of immunoglobulin.

156. A recombinant vector comprising the isolated polynucleotide of claim 148.

157. The recombinant vector of claim 156 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

158. A recombinant host cell comprising the isolated polynucleotide of claim 148.

159. The recombinant host cell of claim 158 wherein the polynucleotide is operably associated with a heterologous regulatory sequence that controls gene expression.

160. A method for producing a protein, comprising: (a) culturing a host cell under conditions suitable to produce a polypeptide encoded by the polynucleotide of claim 148; and (b) recovering the protein from the cell culture.

161. A composition comprising the isolated polynucleotide of claim 148 and a pharmaceutically acceptable carrier.

L11 ANSWER 5 OF 13 USPATFULL on STN
2000:128288 Preparation comprising thiol-group-containing proteins.
Schlag, Guenther, Vienna, Austria
Hallstroem, Seth, Vienna, Austria
Gasser, Harald, Vienna, Austria
Baxter Aktiengesellschaft, Vienna, Austria (non-U.S. corporation)
US 6124255 20000926
APPLICATION: US 1998-8583 19980116 (9)
PRIORITY: AT 1997-68 19970117
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB There is disclosed a stable, virus-safe, pharmaceutical preparation comprising thiol-group-containing proteins which are heat-treated and processed such that at least 40% of the thiol groups are capable of being nitrosated, a method of preparing such preparations as well as the use of these preparations.
CLM What is claimed is:
1. A stable, virus-safe pharmaceutical preparation comprising thiol-group-containing proteins, wherein said thiol-group-containing proteins have been heat-treated and processed such that at least 40% of said thiol groups on said thiol-group-containing proteins are capable of being nitrosated.

2. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins are human proteins.

3. A pharmaceutical preparation according to claim 2, wherein said thiol-group-containing proteins comprises at least one selected from the group consisting of albumin, orosomucoid, tissue-plasminogen activator, fibrinogen, Lys-plasminogen, and hemoglobin.

4. A pharmaceutical preparation according to claim 1, wherein at least 60% of said thiol groups of said thiol-group-containing proteins are nitrosated.

5. A pharmaceutical preparation according to claim 1, wherein 65% to 95% of said thiol groups of said thiol-group-containing proteins are nitrosated.

6. A pharmaceutical preparation according to claim 1, wherein 70% to 90% of said thiol groups of said thiol-group-containing proteins are nitrosated.

7. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins are human S-nitroso-albumin and hemoglobin.

8. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins in said preparation have an N,O,C-nitrosation level that is less than 30%.

9. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins in said preparation have an N,O,C-nitrosation level that is less than 20%.

10. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins in said preparation have an N,O,C-nitrosation level that is less than 10%.

11. A pharmaceutical preparation according to claim 1, wherein said thiol-group-containing proteins have been purified.

12. A pharmaceutical preparation according to claim 11, wherein said thiol-group-containing proteins are at least 80% of the total protein content of the preparation.

13. A pharmaceutical preparation according to claim 11, wherein said thiol-group-containing proteins are at least 90% of the total protein content of the preparation.

14. A pharmaceutical preparation according to claim 4, wherein said pharmaceutical preparation has a stability corresponding to an in vivo half-life of at least 20 minutes.

15. A pharmaceutical preparation according to claim 4, wherein said pharmaceutical preparation has a stability corresponding to an in vivo half-life of 40 minutes to 2 hours.

16. A pharmaceutical preparation according to claim 1, wherein said pharmaceutical preparation is in frozen form.

17. A pharmaceutical preparation according to claim 1, wherein said pharmaceutical preparation is in lyophilized form.

18. A method for preparing a stable, virus-safe pharmaceutical preparation, said method comprising heat treating a composition comprising thiol-group-containing proteins, and processing said thiol-group-containing proteins so as to provide processed proteins with at least 40% of said thiol groups in a form capable of being nitrosated.

19. A method according to claim 18, further comprising nitrosating said thiol groups of said processed proteins with a nitrosating agent.

20. A method according to claim 18, wherein said processing is treating said thiol-group-containing proteins with a reducing agent.

21. A method according to claim 20, wherein said reducing agent is a monothiol-group-containing compound.

22. A method according to claim 20, wherein said reducing agent is β-mercapto-ethanol.

23. A method according to claim 18, wherein said processing is performed at a temperature below 20° C.

24. A method according to claim 18, wherein said processing is performed at a temperature between 2° C. to 100° C.

25. A method according to claim 18, wherein said processing is performed for 1 to 100 hours.

26. A method according to claim 18, wherein said processing is performed for 12 to 48 hours.

27. A method according to claim 18, wherein said composition is selected from the group consisting of plasma, serum, a plasma fraction and a purified protein preparation.

28. A method according to claim 18, further comprising at least one step selected from the group consisting of precipitation, gel filtration, ultrafiltration and chromatographic purification.

29. A method according to claim 18, further comprising at least one purification step after said processing.

30. A method according to claim 19, further comprising at least one purification step after said nitrosating of said thiol groups.

31. A method according to claim 29, wherein said purification step is chromatographic purification.

32. A method according to claim 19, wherein said nitrosating of said thiol groups is carried out under aerobic and acidic conditions with an agent selected from the group consisting of HNO_2, HNO, NOCl, NO+, RNO_2, N_2O_3, N_2O_4, NO_2 -- radical and NO-- radical.

33. A method according to claim 19, wherein said nitrosating is performed by adding a nitrosating agent in the range of 0.5 to 6 parts agent per part thiol-group-containing protein based upon thiol group content.

34. A method according to claim 19, wherein said nitrosating is performed by adding a nitrosating agent in the range of 1 to 2 parts agent per part thiol-group-containing protein based upon thiol group content.

L11 ANSWER 6 OF 13 USPATFULL on STN
2000:4924 Peptide variants of protein A.
Braisted, Andrew C., San Francisco, CA, United States
Starovasnik, Melissa A., Burlingame, CA, United States
Wells, James A., Burlingame, CA, United States
Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
US 6013763 20000111
APPLICATION: US 1996-657983 19960604 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Z domain variants of staphylococcal protein A have significantly reduced size but possess IgG-binding affinity equivalent to the wild type Z domain. These Z domain variants are suitable for use in affinity chromatography purification of proteins and in the treatment of staphylococcic diseases.
CLM What is claimed is:
1. A compound represented by Formula (I) (SEQ ID NO:1)

X_1 -AA_6 -AA_7 -AA_8 -AA_9 -Gln-Gln-AA_{12} -AA_{13} -Phe-Tyr- (I)
- Glu-Ala-Leu-His-Asp-Pro-Asn-Leu-Asn-Glu-Glu-
- Gln-Arg-Asn-Ala-Lys-Ile-AA_{33} -Ser-Ile-AA_{36} -Asp-
- Asp-X_2

where X_1 is selected from the group consisting of H, C_1 -C_6 alkanoyl, and Z-Ala-Val-AA_3 -AA_4 -AA_5 ; where Z is selected from the group consisting of H and C_1 -C_6 alkanoyl; AA_3 is selected from the group consisting of Asp, Arg, and Ala; AA_4 is selected from the group consisting of Asn and Gln; and AA_5 is selected from the group consisting of Lys, Gly, and Ser; AA_6 is selected from the group consisting of Phe and Gly; AA_7 is selected from the group consisting of Asn and Trp; AA_8 is selected from the group consisting of Lys and Met; AA_9 is selected from the group consisting of Glu, Gln, and Arg; AA_{12} is selected from the group consisting of Asn, Ala, and Arg; AA_{13} is selected from the group consisting of Ala and Arg; AA_{33} is selected from the group consisting of Gln and Lys; AA_{36} is selected from the group

consisting of Lys and Arg; and X_2 is selected from the group consisting of OR_1 and NR_1R_2 where R_1 and R_2 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_6-C_{12} aryl and C_6-C_{12} aryl-C_1-C_6 alkyl.

2. The compound of claim 1 selected from the group consisting of Z-Ala-Val-Asp-Asn-Lys-Phe-Asn-Lys-Glu-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-Ala-Leu-His- (SEQ ID NO:5)

Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-Asp-Asp-X.sub.2 ;

Z-Ala-Val-Arg-Asn-Gly-Phe-Asn-Lys-Glu-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-Ala-Leu-His- (SEQ ID NO:6)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-Asp-Asp-X.sub.2 ;

- Z-Ala-Val-Ala-Gln-Ser-Phe-Asn-Lys-Glu-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-Ala-Leu-His- (SEQ ID NO:7)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-Asp-Asp-X.sub.2 ;

- Z-Ala-Val-Asp-Asn-Lys-Phe-Asn-Met-Gln-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-Ala-Leu-His- (SEQ ID NO:8)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-Asp-Asp-X.sub.2 ;

- Z-Ala-Val-Asp-Asn-Lys-Gly-Trp-Met-Arg-Gln-Gln-Asn-Arg-Phe-Tyr-Glu-Ala-Leu-His- (SEQ ID NO:9)

- Asp-Pro-Asn-Leu-Asn-Glu-Glu-Gln-Arg-Asn-Ala-Lys-Ile-Gln-Ser-Ile-Lys-Asp-Asp-X.sub.2 ;

- Z-Ala-Val-Asp-Asn-Lys-Phe-Asn-Lys-Glu-Gln-Gln-Arg-Arg-Phe-Tyr-Glu-Ala-Leu-His- (SEQ ID NO:10)

Asp-Pro-Asn-Leu-Asn-Glu

u-

As

e-

Ly

su

Z-Ala-Val-

Ph

u-

Al

u-

Asp-Pro-As

u-

As

e-

Ly

su

Z-Ala-Val-

Ph

u-

Ar

u-

Asp-Pro-As

u-

As

e-

Ar

su

Z-Ala-Val-

Ph

u-

Ar

u-

Asp-Pro-As

u-

Glu-Gln-Arg-

n-Ala-Lys-Il

Gln-Ser-Ile-

s-Asp-Asp-X.

b.2 ;
-

Asp-Asn-Lys-

e-Asn-Lys-Gl

Gln-Gln-Arg-

a-Phe-Tyr-Gl

Ala-Leu-His-
(SEQ ID
NO:11)
-

n-Leu-Asn-Gl

Glu-Gln-Arg-

n-Ala-Lys-Il

Gln-Ser-Ile-

s-Asp-Asp-X.

b.2 ;
-

Asp-Asn-Lys-

e-Asn-Lys-Gl

Gln-Gln-Asn-

g-Phe-Tyr-Gl

Ala-Leu-His-
(SEQ ID
NO:12)
-

n-Leu-Asn-Gl

Glu-Gln-Arg-

n-Ala-Lys-Il

Lys-Ser-Ile-

g-Asp-Asp-X.

b.2 ;
-

Ala-Asn-Gly-

e-Asn-Met-Gl

Gln-Gln-Arg-

g-Phe-Tyr-Gl

Ala-Leu-His-
(SEQ ID
NO:13)
-

n-Leu-Asn-Gl

Glu-Gln-Arg-
As
n-Ala-Lys-Il
e-
Lys-Ser-Ile-
Ar
g-Asp-Asp-X.
su
b.2 ;
- Ala-Gln-Ser-
Z-Ala-Val-
e-Asn-Met-Gl
Ph
Gln-Gln-Arg-
u-
g-Phe-Tyr-Gl
Ar
Ala-Leu-His-
u-
(SEQ ID
NO:14)
- n-Leu-Asn-Gl
Asp-Pro-As
Glu-Gln-Arg-
u-
n-Ala-Lys-Il
As
Lys-Ser-Ile-
e-
g-Asp-Asp-X.
Ar
b.2 ;
su
- X_1
-Phe-Asn-Met
ln-Gln-Gln-A
-G
-Arg-Phe-Tyr
rg
lu-Ala-Leu-H
-G
-Asp-Pro-Asn
is
eu-Asn-
-L
ID NO:15)
(SEQ
- n-Arg-Asn-Al
Glu-Glu-Gl
Lys-Ile-Lys-
a-
r-Ile-Arg-As
Se
Asp-X_2
p-
;
- Z-Ala-Val-Ala-Gln-Ser-Phe-Asn-Met-Glu-Gln-Gln-Ala-Arg-Phe-Tyr-Glu-
Ala
-Leu-His-
(SEQ ID
NO:24)
- n-Leu-Asn-Gl
Asp-Pro-As
Glu-Gln-Arg-
u-
n-Ala-Lys-Il
As
Lys-Ser-Ile-
e-
g-Asp-Asp-X.
Ar
b.2 ; and
su
- X_1
-Phe-Asn-Met

-G
ln-Gln-Gln-A
la
-Arg-Phe-Tyr
-G
lu-Ala-Leu-H
is
-Asp-Pro-Asn
-L
eu-Asn- (SEQ
ID NO:25)
-
Glu-Glu-Gl
n-Arg-Asn-Al
a-
Lys-Ile-Lys-
Se
r-Ile-Arg-As
p-
Asp-X_2
;

where Z is selected from the group consisting of H and C_1 -C_6 alkanoyl; X_1 is selected from the group consisting of H and C_1 -C_6 alkanoyl; and X_2 is selected from the group consisting of OR_1 and NR_1R_2 where R_1 and R_2 are independently selected from the group consisting of H, C_1 -C_6 alkyl, C_6 -C_{12} aryl and C_6 -C_{12} aryl-C_1 -C_6 alkyl.

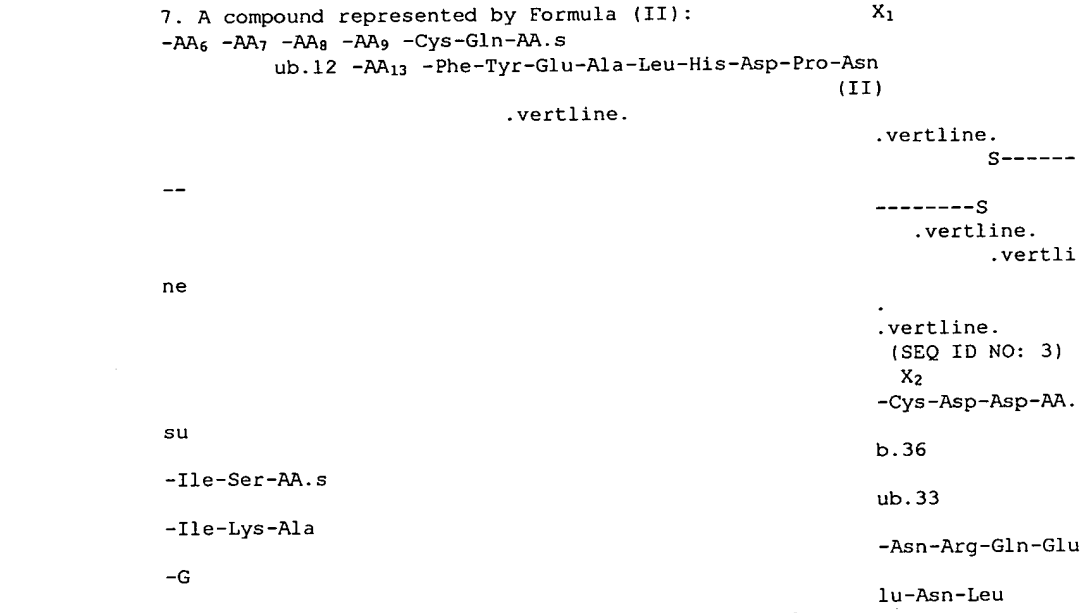
3. The compound of claim 1 that is covalently linked to a macromolecule.

4. The compound of claim 3 wherein the macromolecule is a solid support.

5. The compound of claim 1 that is fused to a selected polypeptide to form a fusion protein.

6. The fusion protein of claim 5 wherein the compound of Formula (I) is specifically cleavable from the selected polypeptide.

7. A compound represented by Formula (II):



where X_1 is selected from the group consisting of H, C_1 -C_6 alkanoyl, and Z-Ala-Val-AA_3 -AA_4 AA_5 (SEQ ID NO:2); where Z is selected from the group consisting of H and C_1 -C_6 alkanoyl; AA_3 is selected from the group consisting of Asp, Arg, and Ala; AA_4 is selected from the group consisting of Asn and Gln; and AA_5 is selected from the group consisting of Lys, Gly, and Ser; AA_6 is selected from the group consisting of Phe and Gly; AA_7 is selected from the group consisting of Asn and Trp; AA_8 is selected from the group consisting of Lys and Met; AA_9 is selected from the group consisting of Glu, Gln, and Arg; AA_{12} is selected from the group consisting of Asn, Ala, and Arg; AA_{13} is selected from the group consisting of Ala and Arg; AA_{33} is selected from the group consisting of Gln and Lys; AA_{36} is selected from the

group consisting of Lys and Arg; and X_2 is selected from the group consisting of OR_1 and NR_1 R_2 where R_1 and R_2 are independently selected from the group consisting of H, C_1 -C_6 alkyl, C_6 -C_{12} aryl and C_6 -C_{12} aryl-C_1 -C_6 alkyl.

8. The compound of claim 7 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Arg; and AA_5 is Gly.

9. The compound of claim 8 wherein AA_4 is Asn; AA_6 is Phe; AA_7 is Asn; AA_8 is Lys; AA_9 is Glu; AA_{12} is Asn; AA_{13} is Arg; AA_{33} is Gln; and AA_{36} is Lys.

10. The compound of claim 7 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Ala; AA_4 is Gln; and AA_5 is Ser.

11. The compound of claim 10 wherein AA_6 is Phe; AA_7 is Asn; AA_8 is Lys; AA_9 is Glu; AA_{12} is Asn; AA_{13} is Arg; AA_{33} is Gln; and AA_{36} is Lys.

12. The compound of claim 7 wherein AA_8 is Met and AA_9 is Gln.

13. The compound of claim 12 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Asp; AA_4 is Asn; AA_5 is Lys; AA_6 is Phe; AA_7 is Asn; AA_{12} is Asn; AA_{13} is Arg; AA_{33} is Gln; and AA_{36} is Lys.

14. The compound of claim 7 wherein AA_6 is Gly; AA_7 is Trp; AA_8 is Met; and AA_9 is Arg.

15. The compound of claim 14 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Asp; AA_4 is Asn; AA_5 is Lys; AA_{12} is Asn; AA_{13} is Arg; AA_{33} is Gln; and AA_{36} is Lys.

16. The compound of claim 7 wherein AA_{12} is Arg.

17. The compound of claim 16 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Asp; AA_4 is Asn; AA_5 is Lys; AA_6 is Phe; AA_7 is Asn; AA_8 is Lys; AA_9 is Glu; AA_{13} is Arg; AA_{33} is Gln; and AA_{36} is Lys.

18. The compound of claim 7 wherein AA_{12} is Arg and AA_{13} is Ala.

19. The compound of claim 18 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Asp; AA_4 is Asn; AA_5 is Lys; AA_6 is Phe; AA_7 is Asn; AA_8 is Lys; AA_9 is Glu; AA_{33} is Gln; and AA_{36} is Lys.

20. The compound of claim 7 wherein AA_{33} is Lys and AA_{36} is Arg.

21. The compound of claim 20 wherein X_1 is Z-Ala-Val-AA_3 -AA_4 -AA_5 (SEQ ID NO:2); AA_3 is Asp; AA_4 is Asn; AA_5 is Lys; AA_6 is Phe; AA_7 is Asn; AA_8 is Lys; AA_9 is Glu; AA_{12} is Asn; and AA_{13} is Arg.

22. The compound of claim 8 wherein AA_8 is Met; AA_9 is Gln; AA_{12} is Arg; AA_{33} is Lys; and AA_{36} is Arg.

23. The compound of claim 22 wherein AA_4 is Asn; AA_6 is Phe; AA_7 is Asn; and AA_{13} is Arg.

24. The compound of claim 10 wherein AA_8 is Met; AA_9 is Gln; AA_{12} is Arg; AA_{33} is Lys; and AA_{36} is Arg.

25. The compound of claim 24 wherein AA_6 is Phe; AA_7 is Asn; and AA_{13} is Arg.

26. The compound of claim 7 wherein X_1 is H or C_1 -C_6 alkanoyl; AA_8 is Met; AA_9 is Gln; AA_{12} is Arg; AA_{33} is Lys; and AA_{36} is Arg.

27. The compound of claim 26 wherein AA_6 is Phe; AA_7 is Asn; and AA_1 3 is Arg.

L11 ANSWER 7 OF 13 USPATFULL on STN
1999:137461 Platelet substitutes and conjugation methods suitable for their preparation.
Heath, David, Nottingham, United Kingdom
Middleton, Sarah Margaret, Nottingham, United Kingdom
Harris, Roy, Nottingham, United Kingdom
Church, Nicola Jane, Nottingham, United Kingdom
Quadrant Healthcare Limited, United Kingdom (non-U.S. corporation)
US 5977313 19991102
APPLICATION: US 1997-953514 19971017 (8)
PRIORITY: GB 1996-21886 19961010
GB 1997-2652 19970210
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Platelet substitutes, comprising fibrinogen, or analogous products useful in therapy, which further comprise an insoluble carrier to which is bound an essentially non-degraded active protein including the sequence Arg-Gly-Asp. Such conjugates can be made by a conjugation process comprising 0.01 to 2.5% by weight active fibrinogen, and no more that 50% inactive fibrinogen.

CLM What is claimed is:

1. A pharmaceutically-acceptable product comprising an insoluble carrier to which is bound an essentially non-degraded active protein selected from fibrinogen and fragments thereof having platelet aggegation activity.

2. A product according to claim 1, wherein the binding is by adsorption.

3. A product according to claim 1, wherein the binding is covalent, via a chemical linker, in the absence of surfactant.

4. A product according to claim 1, wherein the binding is covalent, via a chemical linker at least 10 nm long.

5. A product according to claim 1, further comprising a bound cytotoxic agent.

6. A product according to claim 1, wherein the carrier comprises cross-linked protein microparticles.

7. A product according to claim 6, wherein the protein of the microparticles is albumin.

8. A product according to claim 1, wherein the fibrinogen is full-length.

9. A product according to claim 8, which comprises 0.01 to 2.5% by weight active fibrinogen, and no more than 50% inactive fibrinogen.

10. A method for preparing a covalently-bound conjugate of the formula X--S--Y--Z, wherein X is a carrier component, S is a sulfur atom, Y is a spacer and Z is an active component, which comprises the steps; (i) reacting the active component with a bifunctional reagent of the formula Y^1 --Y--Y^2, wherein Y^1 is a group selectively reactive with free thiol groups and Y^2 is a group reactive with the active component but not with the thiol groups; and (ii) reacting the resultant Y^1 --Y--Z with the carrier component having free thiol groups.

11. A method according to claim 10, wherein Y^2 is COOH.

12. A method according to claim 10, wherein the spacer comprises a fatty acid or peptide chain.

13. A method according to claim 10, wherein the active component has NH_2 groups.

14. A method according to claim 10, wherein the spacer is 10 to 600 nm long.

15. A method according to claim 10, wherein the carrier component is in the form of microparticles.

16. A method according to claim 10, wherein Y^1 is I.

17. A method according to claim 16, wherein the bifunctional reagent is

obtained by reaction of a spacer component with activated iodoacetic acid.

18. A method according to claim 10, wherein the active component is a protein including the sequence Arg-Gly-Asp.

19. A method according to claim 18, wherein the protein is fibrinogen.

20. A method according to claim 10, wherein the carrier is albumin.

21. Albumin conjugated via its thiol groups to an active component by means of a spacer at least 50 nm long produced by the method according to claim 20.

22. A method according to claim 20, wherein the carrier is human **serum albumin**.

23. Albumin conjugated via its thiol groups to an active component by means of a spacer at least 50 nm long produced by the method of claim 22.

24. Albumin conjugated via its thiol groups to an active component selected from fibrinogen and fragments thereof having platelet aggregation activity, by means of a spacer at least 50 nm long.

25. Albumin according to claim 24 wherein the albumin is in the form of microparticles.

26. Albumin conjugated according to claim 21, further comprising a bound cytotoxic agent.

27. Albumin according to claim 24, wherein the fibrinogen is full-length, for use as a platelet substitute.

28. Albumin according to claim 27, wherein the fibrinogen comprises 0.01 to 2.5% by weight active fibrinogen, and no more than 50% inactive fibrinogen.

L11 ANSWER 8 OF 13 USPATFULL on STN
1998:82871 Biologically active protein fragments containing specific binding regions of **serum albumin** or related proteins.
Carter, Daniel C., Huntsville, AL, United States
The United States of America as represented by the Administrator of the National Aeronautics and Space Administration, Washington, DC, United States (U.S. government)
US 5780594 19980714
APPLICATION: US 1995-448196 19950523 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB In accordance with the present invention, biologically active protein fragments can be constructed which contain only those specific portions of the **serum albumin** family of proteins such as regions known as subdomains IIA and IIIA which are primarily responsible for the binding properties of the serum albumins. The artificial serums that can be prepared from these biologically active protein fragments are advantageous in that they can be produced much more easily than serums containing the whole albumin, yet still retain all or most of the original binding potential of the full albumin proteins. In addition, since the protein fragment serums of the present invention can be made from non-natural sources using conventional recombinant DNA techniques, they are far safer than serums containing natural albumin because they do not carry the potentially harmful viruses and other contaminants that will be found in the natural substances.

CLM What is claimed is:
1. A **serum albumin** protein fragment consisting of at least one **serum albumin** binding region selected from the group consisting of binding region subdomain IIA and binding region subdomain IIIA.

2. A **serum albumin** protein fragment according to claim 1 wherein the **serum albumin** binding region consists of binding region subdomain IIA.

3. A **serum albumin** protein fragment according to claim 1 wherein the **serum albumin** binding region consists of binding region subdomain IIIA.

4. A **serum albumin** protein fragment according to claim 1 wherein the **serum albumin** binding region consists of binding region subdomains IIA, IIB and IIIA.

5. A **serum albumin** protein fragment according to claim 1 wherein the **serum albumin** binding region is a binding region of a **serum albumin** selected from the group consisting of human, bovine, equine, ovine, rat, frog, sheep, salmon, mouse, and sea lamprey **serum albumin** proteins.

6. A **serum albumin** protein fragment according to claim 5 wherein the **serum albumin** binding region is a human **serum albumin** binding region.

7. A **serum albumin** protein fragment according to claim 5 wherein the **serum albumin** binding region is an equine **serum albumin** binding region.

8. A **serum albumin** protein fragment according to claim 5 wherein the **serum albumin** binding region is a bovine **serum albumin** binding region.

9. A **serum albumin** protein fragment according to claim 8 wherein the **serum albumin** binding region consists of SEQ ID NO: 1.

10. A **serum albumin** protein fragment according to claim 8, wherein the **serum albumin** binding region consists of SEQ ID NO:2.

11. A **serum albumin** protein fragment according to claim 4 wherein the **serum albumin** binding region consists of amino acids 190 to 494 of SEQ ID NO:4.

L11 ANSWER 9 OF 13 USPATFULL on STN
97:112148 Magnetic resonance blood pool agents bound to human **serum albumin**

Moore, Dennis A., Ferguson, MO, United States
Cooper, Stephen R., St. Louis, MO, United States
Wallace, Rebecca Abernathy, Manchester, MO, United States
Hynes, Michael R., Florissant, MO, United States
Mallinckrodt Medical, Inc., St. Louis, MO, United States (U.S. corporation)
US 5693308 19971202
APPLICATION: US 1996-604286 19960221 (8)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides compositions comprising a contrast agent capable of reversibly binding to thiol groups of blood-borne proteins. The compositions of the invention provide increased residence time of the contrast agent in the vasculature, thus providing effective blood pool contrast agents. The invention also provides methods for imaging a patient comprising administering a composition of the invention and obtaining an image.

CLM What is claimed:
1. A diagnostic composition comprising a contrast agent containing a thiol or disulfide through which it is reversibly bound to a thiol or disulfide moiety of human **serum albumin**.

2. The diagnostic composition of claim 1 wherein the thiol moiety on the contrast agent is selected from mercaptoacetyl, 2-mercaptoaminoethyl, mercaptobenzoyl, 3-mercaptopropionyl and thiosalicyl.

3. The composition of claim 1 wherein the thiol moiety is mercaptoacetyl.

4. The composition of claim 1 wherein the thiol moiety is 2-mercaptoaminoethyl.

5. The composition of claim 1 wherein the thiol moiety is 3 thiosalicyl.

6. The composition of claim 1 wherein the disulfide moiety on the contrast agent is selected from dithiodiacetyl, dithiodiacetacarbamoyl, 2,2'-dithiodiaminoethyl, dithiodibenzoyl, 3,3'-dithiodiproprionyl and dithiodisalicyl.

7. The composition of claim 6 wherein the disulfide moiety is dithiodiacetyl.

8. The composition of claim 6 wherein the disulfide moiety is dithiodibenzoyl.

9. The composition of claim 6 wherein the disulfide moiety is 3,3'-dithiodiproprionyl.

10. A method of imaging a patient comprising the administration of a diagnostically effective amount of a composition comprising a contrast agent containing a thiol or disulfide reversibly bound to a thiol or

disulfide moiety of human **serum albumin**, and obtaining an image.

11. The method of claim 10 wherein the disulfide on the contrast agent is selected from dithiodiacetyl, dithiodiacetacarbamoyl, 2,2'-dithiodiaminoethyl, dithiodibenzoyl, 3,3'-dithiodiproprionyl and dithiodisalicyl.

12. The method of claim 11 wherein the disulfide moiety is dithiidoacetyl.

13. The method of claim 11 wherein the disulfide moiety is dithiodibenzoyl.

14. The method of claim 11 wherein the disulfide moiety is 3,3'-dithiodiproprionyl.

15. The method of claim 10 wherein the thiol moiety on the contrast agent is selected from mercaptoacetyl, 2-mercaptoaminoethyl, mercaptobenzoyl, 3-mercaptopropionyl and thiosalicyl.

16. The method of claim 15 wherein the thiol moiety is mercaptoacetyl.

17. The method of claim 15 wherein the thiol moiety is 2-mercaptoaminoethyl.

18. The method of claim 15 wherein the thiol moiety is thiosalicyl.

L11 ANSWER 10 OF 13 USPATFULL on STN
97:73720 Oligosaccharide oxazolines, oligosaccharide conjugates and methods of preparation thereof.
Colon, Marcelo, BO. Jaquas BZ 280, Gurabo, Puerto Rico 00658
Davis, Jeffrey T., 14 Hovey St., Watertown, MA, United States 02172
Rasmussen, James R., 75-83 Cambridge Pkwy. E411, Cambridge, MA, United States 02142
Borowski, Marianne, 437 Marlborough St., Boston, MA, United States 02115
Wan, Barbara Y., 83 Willow St., Tewksbury, MA, United States 01876
Hirani, Shirish, 130 Dartmouth St., #703, Boston, MA, United States 02116
US 5659015 19970819
APPLICATION: US 1992-959701 19921013 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to a method of producing peracetyloxazolines from peracetyl saccharides. The method involves reacting the starting material, a peracetyl saccharide, with a reagent combination, to directly produce the peracetyl oxazoline. This method may be used for the activation of oligosaccharides, wherein an oligosaccharide containing a reducing GlcNAc terminus is activated by the formation of an oxazolide at the terminal GlcNAc, and then coupled with a bifunctional spacer to provide an oligosaccharide-spacer conjugate. The activated oligosaccharide-spacer conjugate is then coupled to a protein, such as granulocyte colony stimulating factor or γ-interferon, providing a neoglycoprotein conjugate. The invention provides a method for forming neoglycoprotein conjugates which may improve biological and physiochemical properties of the protein. For example, serum lifetime or efficiency of drug delivery of the peptide to a target organ or cell may be improved.
CLM What is claimed is:
1. An oligosaccharide-protein conjugate with an oligosaccharide having a terminal 2-acetamido-2-deoxyglucopyranose (GlcNAc) capable of forming a peracetyl oxazoline, the GlcNAc being bound to a bifunctional spacer at a carbon on the terminal GlcNAc so as to form a β-O-glycosidic bond, the bifunctional spacer being coupled to a cysteine on a protein, wherein the bifunctional spacer has the formula --O--$(CH_2)_n$ --X--Y--$(CH_2)_n$ --Z, such that n is an integer in the range of 1 to 10, and X and Y are selected from the group consisting of an amide, an aliphatic hydrocarbon, a carbonyl group and a thioester and Z is selected from the group consisting of --CH_2 and --N.

2. A conjugate according to claim 1, wherein the bifunctional spacer has the formula: ##STR9##

3. A conjugate according to claim 1, wherein the oligosaccharide includes a structure of the formula $(Mannose)_n$ -2-acetamido-2-deoxyglucopyranose and n is an integer in the range from 3 to 9.

4. A conjugate according to claim 1, wherein the protein is γ-interferon.

5. A conjugate according to claim 1, wherein the oligosaccharide includes a structure of the formula Man_n -GlcNAc n being an integer in the range from 3 to 9, the protein is γ-interferon, and the bifunctional spacer has the formula --O--$(CH_2)_5$ --NH--C--CH_2 --.

6. A conjugate between an oligosaccharide having a reducing GlcNAc terminus and a protein having a nucleophile coupled by a bifunctional spacer having the formula: Os-A-B-C-Nu-Pr, wherein: Os is the oligosaccharide with a terminal GlcNAc; A is β-O--; B is a spacer; C is the product of the reaction between the electrophilic group on the oligosaccharide-spacer conjugate with the nucleophilic group on the protein; Nu is a nucleophilic group on the protein; and Pr is the protein.

7. A conjugate according to claim 6, wherein the spacer B has the formula --$(CH_2)_n$ --X--Y--$(CH_2)_n$ --Z -- wherein: n is an integer in the range from 1 to 10; and X and Y are selected from the group consisting of a first amide, a first aliphatic hydrocarbon, a carbonyl group and a thioester and Z is selected from the group consisting of a second aliphatic hydrocarbon and a second amide.

8. A conjugate according to claim 7 wherein spacer B is selected from the group consisting of --$(CH_2)_n$ --CO--NH--$(CH_2)_n$ --Z, --$(CH_2)_n$ --NH--CO--$(CH_2)_n$ --Z, --$(CH_2)_n$ --S--CO--$(CH_2)_n$ --Z, --$(CH_2)_n$ --CO--S--$(CH_2)_n$ --Z, and --$(CH_2)_n$ --CH_2 --CH_2 --$(CH_2)_n$ --Z, where --Z is --CH_2 or --N.

9. A conjugate according to claim 7, wherein the group C has the formula: ##STR10##

10. A conjugate according to claim 9, wherein the spacer has the formula: ##STR11##

11. A conjugate, according to claim 7, wherein the oligosaccharide includes a structure of the formula Man_n -GlcNAc where n is an integer in the range from 3 to 9.

12. A conjugate according to claim 9, wherein the protein is γ-interferon.

13. A conjugate according to claim 9, wherein the oligosaccharide includes a structure of the formula Man_n -GlcNAc and n is an integer in the range from 3 to 9, the protein is γ-interferon, and the bifunctional spacer has the formula --O--$(CH_2)_5$ --NH--C--CH_2 --.

14. A method for obtaining an oligosaccharide-protein conjugate comprising: (a) obtaining an oligosaccharide having a reducing GlcNAc terminus; (b) forming an oxazoline at the GlcNAc terminus; (c) using the oxazoline for conjugating a bifunctional spacer to a carbon on the GlcNAc terminus so as to form a β-O-glycosidic bond between the oligosaccharide and the spacer; and (d) coupling the oligosaccharide-spacer conjugate to a cysteine on a protein.

15. A method for obtaining a conjugate according to claim 14, wherein the bifunctional spacer used in step (c) has the formula: ##STR12##

16. A method for obtaining a conjugate according to claim 14, wherein the oligosaccharide obtained in step (a) includes a structure of the formula Man_n GlcNAc and n is an integer in the range from 3 to 9.

17. A method for obtaining a conjugate according to claim 14, wherein the protein used in step (d) is γ-interferon.

18. A method for obtaining a conjugate, according to claim 14, wherein the oligosaccharide obtained in step (a) includes a compound of the formula Man_n -GlcNAc, n being an integer in the range from 3-9, the protein used in step (d) is gamma interferon and the bifunctional spacer used in step (c) has the formula O--$(CH_2)_5$ --NH--C--CH_2 --.

19. A method for the synthesis of a glycopeptide comprising: (a) selecting a reagent combination having 2 or more reagents, suitable for reacting with a peracetyl saccharide so as to protect the reactants from

concomitant glycosidic cleavage, the reagent combination being capable of forming a peracetyl oxazoline from the peracetyl saccharide; (b) reacting the peracetyl saccharide with the reagent combination so as to form the peracetyl oxazoline; (c) reacting the peracetyl oxazoline with an azide to produce a glycosyl azide; (d) reducing the glycosyl azide to produce a glycosyl amine; and (e) coupling the glycosyl amine with an activated carboxylic group in an amino acid or peptide.

20. A method for the synthesis of a glycopeptide comprising: (a) selecting a reagent combination having 2 or more reagents, suitable for reacting with a saccharide derivative selected from the group consisting of a pertrichloroacetyl, a pertrifluoroacetyl and a perbenzoyl saccharide so as to protect the reactants from concomitant glycosidic cleavage, the reagent combination being capable of forming a peracetyl oxazoline from said saccharide derivative; (b) reacting the saccharide derivative with the reagent combination so as to form the peracetyl oxazoline; (c) reacting the peracetyl oxazoline with an azide to produce a glycosyl azide; (d) reducing the glycosyl azide to produce a glycosyl amine; and (e) coupling the glycosyl amine with an activated carboxylic group in an amino acid or peptide.

21. A conjugate between an oligosaccharide having a reducing GlcNAc terminus and a protein having a nucleophile coupled by a bifunctional spacer having the formula: Os-A-B-C-Nu-Pr, wherein: Os is the oligosaccharide with a terminal GlcNAc; A is β-N--(H)--CO--; B is a spacer; C is the product of the reaction between the electrophilic group on the oligosaccharide-spacer conjugate and the nucleophilic group on the protein; Nu is a nucleophilic group on the protein; and Pr is the protein.

L11 ANSWER 11 OF 13 USPATFULL on STN
95:84455 Antagonists of human gamma interferon.
Seelig, Gail F., Watchung, NJ, United States
Schering Corporation, Kenilworth, NJ, United States (U.S. corporation)
US 5451658 19950919
WO 9206115 19920416
APPLICATION: US 1993-30077 19930319 (8)
WO 1991-US6771 19910925 19930319 PCT 371 date 19930319 PCT 102(e) date
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Novel synthetic polypeptides having amino acid sequences corresponding to the sequence of one or more specific regions of human gamma interferon are provided by this invention. These polypeptides specifically inhibit the binding of human gamma interferon to cellular receptors and the biological activity of such interferon. Antibody antagonists of the binding of human gamma interferon to cellular receptors based Upon the use of the polypeptides as antigens are also provided. Some of these antagonists bind to specific regions of gamma interferon which are believed to be involved in interactions between the intefferon and its receptors. Other antibody antagonists are anti-idiotypic antibodies which appear to compete directly with gamma interferon for binding to the cellular receptors. Also provided are methods for the use of the polypeptides and antibodies as inhibitors of the binding of gamma interferon to its cellular receptors.
CLM What is claimed is:
1. A polypeptide which has an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:10.

L11 ANSWER 12 OF 13 USPATFULL on STN
93:72218 Oligosaccharide oxazolines, oligosaccharide conjugates and methods of preparation thereof.
Colon, Marcelo, Gurabo, PR, United States
Davis, Jeffrey T., Watertown, MA, United States
Rasmussen, James R., Cambridge, MA, United States
Borowski, Marianne, Boston, MA, United States
Wan, Barbara Y., Tewksbury, MA, United States
Hirani, Shirish, Boston, MA, United States
Genzyne Corporation, United States (U.S. corporation)
US 5241072 19930831
APPLICATION: US 1990-529343 19900525 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to a method of producing peracetyloxazolines from peracetyl saccharides. The method involves reacting the starting material, a peracetyl saccharide, with a reagent combination, to directly produce the peracetyl oxazoline. This method may be used for the activation of oligosaccharides, wherein an oligosaccharide

containing a reducing GlcNAc terminus is activated by the formation of an orazoline at the terminal GlcNAc, and then coupled with a bifunctional spacer to provide an oligosaccharide-spacer conjugate. The activated oligosaccharide-spacer conjugate is then coupled to a protein, such as granulocyte colony stimulating factor or γ-interferon, providing a neoglycoprotein conjugate. The invention provides a method for forming neoglycoprotein conjugates which may improve biological and physiochemical properties of the protein. For example, serum lifetime or efficiency of drug delivery of the peptide to a target organ or cell may be improved.

CLM What is claimed is:

1. A process for the production of a peracetyl saccharide oxazoline comprising reacting a peracetyl saccharide having a reducing terminus with a 2-N-acetyl group, with reagents consisting essentially of boron trifluoride etherate and a compound selected from the group consisting of bromotrimethylsilane, chlorotrimethylsilane and iodotrimethylsilane.

2. A method for the production of a peracetyl oxazoline according to claim 1, wherein the peracetyl saccharide is selected from mono-, di-, and oligosaccharides.

3. A method for the production of an oxazoline according to claim 1, wherein the saccharide may be a pertrichloroacetyl, pertrifluoroacetyl or perbenzoylacetyl saccharide.

4. A method for the production of a peracetyl oxazoline according to claim 1, wherein the reagents are composed of a 1:1 mixture of boron trifluoride etherate and the compound.

5. A method for the production of a peracetyl oxazoline according to claim 1, wherein an acid scavenger is added which is selected from the group of acid scavengers consisting of 2,4,6-collidine, pyridine, triethylamine, dimethylaminopyridine and N,N-diisopropylethylamine.

6. A process for the production of a peracetyl saccharide oxazoline comprising reacting a peracetyl saccharide having a reducing terminus and a 2-N-acetyl group, with reagents consisting essentially of boron trifluoride etherate and a compound selected from the group consisting of bromotrimethylsilane and chlorodimethylsilane.

L11 ANSWER 13 OF 13 USPATFULL on STN

93:69984 Proteins having anticoagulant properties.

Vlasuk, George P., Lansdale, PA, United States
Waxman, Lloyd H., Ottsville, PA, United States
Garsky, Victor M., Blue Bell, PA, United States
Neeper, Michael P., Wayne, PA, United States
Merck & Co., Inc., Rahway, NJ, United States (U.S. corporation)

US 5239058 19930824
APPLICATION: US 1992-876825 19920429 (7)
DOCUMENT TYPE: Utility; Granted.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Proteins which specifically inhibit coagulation Factor Xa. The inhibitors, which do not inhibit Factor VIIa, kallikrein, trypsin, chymotrypsin, thrombin, urokinase, tissue plasminogen activator, plasmin, elastase, Factor XIa or S. aureus V8 protease, are polypeptides of 60 amino acid residues. The inhibitors may be purified from Ornithodoros moubata extract, synthesized, or produced using a recombinant DNA yeast expression system.

CLM What is claimed is:

1. A purified and isolated protein having the sequence: ##STR6## wherein AA^{9} is Arg or Asn, AA^{23} is Arg or Asn, AA^{27} is Arg or Asn, and AA^{53} is Arg or Asn.

2. A protein of claim 1, wherein AA^{9} is Arg, AA^{23} is Arg, AA^{27} is Arg, and AA^{53} is Arg.

3. A protein of claim 1, wherein AA^{9} is Asn, AA^{23} is Arg, AA^{27} is Arg, and AA^{53} is Arg.

4. A protein of claim 1, wherein AA^{9} is Arg, AA^{23} is Arg, AA^{27} is Arg, and AA^{53} is Arg.

5. A protein of claim 1, wherein AA^{9} is Arg, AA^{23} is Arg, AA^{27} is Asn, and AA^{53} is Arg.

6. A protein of claim 1, wherein AA^{9} is Arg, AA^{23} is Arg, AA^{27} is Arg, and AA^{53} is Asn.

7. A therapeutic composition for inhibiting blood coagulation Factor Xa comprising an effective amount of the protein of claim 1 and a pharmaceutically-acceptable carrier.

8. A method for inhibiting blood coagulation Factor Xa in a mammal comprising administering to the mammal an effective dose of a composition of claim 7.

9. A method for achieving thrombolytic reperfusion following thrombus formation in a patient comprising administering a protein of claim 1 to inhibit Factor Xa and thereafter administering tissue plasminogen activator or other plasminogen activator.

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=> d his

     (FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

     FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005
L1         68146 S (SERUM ALBUMIN)
L2            74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3            39 S L2 AND PY<2000
L4         15041 S L1 AND PHARMACOLOG?
L5          1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6             2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
L7            47 S L4 AND (MALEIMID?)
L8            28 S L7 AND PY<2000

     FILE 'USPATFULL' ENTERED AT 20:20:48 ON 26 NOV 2005
L9         59637 S (SERUM ALBUMIN)
L10           80 S L9 AND (CYS-34 OR CYSTEINE-34)
L11           13 S L10 AND AY<2000

=> s l9 and (enhanced pharmacokinetic)
        521940 ENHANCED
         15276 PHARMACOKINETIC
            72 ENHANCED PHARMACOKINETIC
                 (ENHANCED(W)PHARMACOKINETIC)
L12           13 L9 AND (ENHANCED PHARMACOKINETIC)

=> s l12 not l10
L13           11 L12 NOT L10

=> d l13,cbib,ab,clm,1-11

L13  ANSWER 1 OF 11  USPATFULL on STN
2005:164675 Polymer conjugates of mutated neublastin.
    Sah, Dinah Wen-Yee, Boston, MA, UNITED STATES
    Pepinsky, R. Blake, Arlington, MA, UNITED STATES
    Boriack-Sjodin, Paula Ann, Waltham, MA, UNITED STATES
    Miller, Stephan S., Arlington, MA, UNITED STATES
    Rossomando, Anthony, Revere, MA, UNITED STATES
    Silvian, Laura, Waban, MA, UNITED STATES
    US 2005142098 A1 20050630
    APPLICATION: US 2003-356264  A1  20030131 (10)
    PRIORITY: US 2001-266071P  20010201 (60)
    DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB     A dimer comprising a mutated neublastin polypeptide coupled to a polymer
       is disclosed. Such dimers exhibit prolonged bioavailability and, in
       preferred embodiments, prolonged biological activity relative to
       wild-type forms of neublastin.
CLM    What is claimed is:
       1. A polymer-conjugated dimer comprising a first polypeptide comprising
       a first amino-terminal amino acid and a second polypeptide comprising a
       second amino-terminal amino acid, wherein each polypeptide individually
       comprises:  a) an amino acid sequence characterized by at least 70%
       sequence identity with amino acids 8-113 of SEQ ID NO:1;  b) a cysteine
       residue at each of positions 16, 43, 47, 80, 81, 109, and 111 when the
       polypeptides are numbered in accordance with SEQ ID NO:1;  c) amino acid
       residues as follows:  C at position 16, L at position 18, V at position
       25, L at position 28, G at position 29, L at position 30, G at position
       31, E at position 36, F at position 40, R at position 41, F at position
       42, C at position 43, G at position 45, C at position 47, C at position
       80, C at position 81, R at position 82, P at position 83, F at position
       91, D at position 93, S at position 105, A at position 106, C at
       position 109 and C at position 111, each when numbered in accordance
       with SEQ ID NO:1; and  d) an LGLG repeat, an FRFC motif, a QPCCRP motif,
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and a SATACGC motif; wherein at least the first polypeptide comprises at least one amino acid substitution, insertion or fusion as compared to SEQ ID NO:1, and wherein the substitution, insertion or fusion provides an internal polymer conjugation site to which a polymer is conjugated.

2. The dimer of claim 1, wherein the amino acid sequence is characterized by at least 95% sequence identity with amino acids 8-113 of SEQ ID NO:1.

3. A polymer-conjugated dimer comprising a first polypeptide and a second polypeptide, wherein each polypeptide individually comprises 90 to 140 amino acids of SEQ ID NO:6 with 1-6 amino acid substitutions, each substitution providing a polymer conjugation site to which a polymer is conjugated.

4. The dimer of claim 1, wherein the first polypeptide is selected from the group consisting of NBN113, NBN140, NBN116, NBN112, NBN111, NBN110, NBN109, NBN108, NBN107, NBN106, NBN105, NBN104, NBN103, NBN102, NBN101, NBN100 and NBN99 (SEQ ID NOs:2, 6-21, respectively).

5. The dimer of claim 1 or 3, wherein the first amino-terminal amino acid or the second amino-terminal amino acid is conjugated to a polymer.

6. The dimer of claim 5, wherein the first amino-terminal amino acid and the second amino-terminal amino acid are conjugated to a polymer.

7. The dimer of claim 3, wherein at least one substitution is selected from the group consisting of: an amino acid other than arginine at position 14 in the amino acid sequence of said first polypeptide, second polypeptide, or both; an amino acid other than arginine at position 39 in the amino acid sequence of said first polypeptide, second polypeptide, or both; an amino acid other than arginine at position 68 in the amino acid sequence of said first polypeptide, second polypeptide, or both; and an amino acid other than asparagine at position 95 in the amino acid sequence of said first polypeptide, second polypeptide, or both; wherein the positions of said amino acids are numbered in accordance with the polypeptide sequence of SEQ ID NO:1.

8. The dimer of claim 1, wherein the amino acid substitution is a lysine in place of an asparagine.

9. The dimer of claim 3, wherein the amino acid substitution is a lysine in place of an asparagine.

10. The dimer of claim 1, wherein the amino acid substitution is a lysine in place of an arginine.

11. The dimer of claim 3, wherein the amino acid substitution is a lysine in place of an arginine.

12. The dimer of claim 1, wherein the amino acid substitution is a neutral or acidic amino acid in place of an arginine at position 48, 49 or 51.

13. The dimer or claim 12, wherein the neutral or acidic amino acid is selected from the group consisting of glutamate and aspartate.

14. The dimer of claim 9, wherein the substitution is at position 95.

15. The dimer of claim 1, wherein the substitution is an aspartate in place of an asparagine at position 95.

16. The dimer of claim 1 or 2, wherein the average molecular weight of the polymer is about 2000 Da to about 100,000 Da.

17. The dimer of claim 11, wherein the average molecular weight of the polymer is about 5,000 Da to about 50,000 Da.

18. The dimer of claim 12, wherein the average molecular weight of the polymer is about 10,000 Da to about 20,000 Da.

19. The dimer of claim 1 or 3, wherein the polymer is linear.

20. The dimer of claim 1 or 3, wherein the polymer is branched.

21. The dimer of claim 1 or 3, wherein the polymer consists essentially of a polyalkylene glycol moiety.

22. The dimer of claim 16, wherein the polyalkylene glycol moiety is a

PEG moiety.

23. The dimer of claim 1 or 3, wherein at least one polypeptide is glycosylated.

24. A polymer-conjugated dimer comprising a first polypeptide and a second polypeptide, wherein: (a) each polypeptide individually comprises 100 to 110 amino acids of SEQ ID NO:1, (b) each polypeptide comprises an asparagine-to-lysine substitution at amino acid number 95 in SEQ ID NO:1, (c) and the dimer comprises 3 or 4 PEG moieties, wherein the molecular weight of each PEG moiety is about 10,000 Da, and each PEG moiety is conjugated at an amino-terminus or at lysine 95.

25. A composition comprising; a) a homodimer of NBN106-N95K conjugated to three PEG polymers having a molecular weight of about 10,000 Da; b) a homodimer of NBN106-N95K conjugated to four PEG polymers having a molecular weight of about 10,000 Da; or c) a mixture of at least two different dimers of claim 1 or 3.

26. A nucleic acid that encodes the first polypeptide of claim 1 or 3.

27. A host cell transformed with the nucleic acid of claim 21.

28. A method for treating neuropathic pain in a mammal, the method comprising administering to the mammal a therapeutically effective amount of the dimer of claim 1 or 3.

29. The method of claim 28, wherein the therapeutically effective amount is from 0.01 μg/kg to 1000 μg/kg.

30. The method of claim 29, wherein the therapeutically effective amount is from 1 μg/kg to 100 μg/kg.

31. The method of claim 30, wherein the therapeutically effective amount is from 1 μg/kg to 30 μg/kg.

32. The method of claim 31, wherein the therapeutically effective amount is from 3 μg/kg to 10 μg/kg.

33. The method of claim 28, wherein the dimer is administered via intramuscular delivery or subcutaneous delivery.

34. A method of activating the RET receptor in a mammal, the method comprising administering to the mammal an effective amount of the dimer of claim 1 or 3.

L13 ANSWER 2 OF 11 USPATFULL on STN
2005:138820 Conjugated oligomeric compounds and their use in gene modulation.
Manoharan, Muthiah, Weston, MA, UNITED STATES
Baker, Brenda, Carlsbad, CA, UNITED STATES
Eldrup, Anne, Ridgefield, CT, UNITED STATES
Bhat, Balkrishen, Carlsbad, CA, UNITED STATES
Griffey, Richard H., Vista, CA, UNITED STATES
Swayze, Eric E., Carlsbad, CA, UNITED STATES
Crooke, Stanley T., Carlsbad, CA, UNITED STATES
US 2005119470 A1 20050602
APPLICATION: US 2003-700971 A1 20031104 (10)
PRIORITY: US 2002-423760P 20021105 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides modified oligomeric compounds that modulate gene expression via an RNA interference pathway. The oligomeric compounds of the invention include one or more conjugate moieties that can modify or enhance the pharmacokinetic and phamacodynamic properties of the attached oligomeric compound.
CLM What is claimed is:
1. A composition comprising: a first oligomeric compound and a second oligomeric compound, wherein at least a portion of said first oligomeric compound is capable of hybridizing with at least a portion of said second oligomeric compound, wherein at least a portion of said first oligomeric compound is capable of hybridizing to a target nucleic acid, and wherein at least one of said first and said second oligomeric compounds comprises at least one conjugate moiety.

2. The oligonucleotide composition of claim 1 wherein said first and said second oligomeric compounds form a complementary pair of siRNA oligonucleotides.

3. The composition of claim 1 wherein said first and said second oligonucleotides comprise an antisense/sense pair of oligonucleotides.

4. The composition of claim 1 wherein each of said first and second oligomeric compounds comprises 10 to 40 nucleotides.

5. The composition of claim 1 wherein each of said first and second oligomeric compounds comprises 18 to 30 nucleotides.

6. The composition of claim 1 wherein each of said first and second oligomeric compounds comprises 21 to 24 nucleotides.

7. The composition of claim 1 wherein said first oligomeric compound comprises an antisense oligonucleotide.

8. The composition of claim 7 wherein said second oligomeric compound comprises a sense oligonucleotide.

9. The composition of claim 7 wherein said second oligomeric compound comprises an oligonucleotide having a plurality of ribose nucleotide units.

10. The composition of claim 1 wherein said first oligomeric compound comprises said at least one conjugate moiety.

11. The composition of claim 1 wherein said second oligomeric compound comprises said at least one conjugate moiety.

12. The composition of claim 1 wherein said first and second oligomeric compounds each comprises at least one conjugate moiety.

13. The composition of claim 1 wherein said second oligomeric compound comprises at least one conjugate moiety and said first oligomeric compound comprises no conjugate moiety.

14. The composition of claim 13 wherein said second oligomeric compound comprises a sense oligonucleotide.

15. The composition of claim 1 wherein said at least one conjugate moiety is attached to an internal residue of said first or second oligomeric compounds.

16. The composition of claim 1 wherein said at least one conjugate moiety is attached to a terminal residue of said first or second oligomeric compounds.

17. The composition of claim 16 wherein said terminal residue is at the 5' end of said first or second oligomeric compounds.

18. The composition of claim 1 wherein said conjugate moiety is attached to a heterocyclic base moiety of said first or second oligomeric compounds.

19. The composition of claim 1 wherein said at least one conjugate moiety is attached to a monomeric subunit of said first or second oligomeric compounds.

20. The composition of claim 1 wherein said at least one conjugate moiety is attached to a monomeric subunit linkage of said first or second oligomeric compounds.

21. The composition of claim 1 wherein said at least one conjugate moiety is attached to said first or second oligomeric compounds through a linker.

22. The composition of claim 1 wherein said at least one conjugate moiety is a lipophilic moiety, vitamin, polymer, peptide, protein, nucleic acid, small molecule, oligosaccharide, carbohydrate cluster, intercalator, minor groove binder, cleaving agent, or cross-linking agent.

23. The composition of claim 1 wherein said at least one conjugate moiety is a steroid.

24. The composition of claim 1 wherein said at least one conjugate moiety is cholesterol or a cholesterol derivative.

25. The composition of claim 1 wherein said at least one conjugate

moiety binds to low-density lipoprotein.

26. The composition of claim 1 wherein said at least one conjugate moiety is folate or folate derivative.

27. The composition of claim 1 wherein said at least one conjugate moiety is a water-soluble polymer.

28. The composition of claim 1 wherein said at least one conjugate moiety comprises polyethylene glycol or copolymer thereof.

29. The composition of claim 1 wherein said at least one conjugate moiety comprises a fusogenic peptide or delivery peptide.

30. The composition of claim 1 wherein said at least one conjugate moiety comprises a nuclear export signal.

31. The composition of claim 1 wherein said at least one conjugate moiety comprises a nucleic acid.

32. The composition of claim 1 wherein said at least one conjugate moiety comprises a drug.

33. The composition of claim 1 wherein said at least one conjugate moiety binds to human **serum albumin**.

34. The composition of claim 1 wherein said at least one conjugate moiety comprises a reporter group.

35. The composition of claim 1 wherein said at least one conjugate moiety localizes said first oligomeric compound, said second oligomeric compound, or both to the cytoplasm of a cell.

36. The composition of claim 1 wherein said at least one conjugate moiety enhances the pharmacokinetic or pharmacodynamic properties of said composition.

37. The composition of claim 1 wherein said composition has improved cellular uptake properties compared with the same composition having no conjugate moiety.

38. A composition comprising, a first oligomeric compound capable of hybridizing to a target nucleic acid, optionally a second oligomeric compound hybrizable to said first oligomeric compound; at least one protein, said protein comprising at least a portion of a RNA-induced silencing complex (RISC), wherein said composition comprises at least one oligomeric compound comprising at least one conjugate moiety.

39. The composition of claim 38 wherein said first oligomeric compound comprises an antisense oligonucleotide.

40. The composition of claim 38 wherein said first oligomeric compound comprises 10 to 40 nucleotides.

41. The composition of claim 38 wherein said first oligomeric compound comprises 18 to 30 nucleotides.

42. The composition of claim 38 wherein said first oligomeric compound comprises 21 to 24 nucleotides.

43. The composition of claim 38 comprising said second oligomeric compound.

44. The composition of claim 43 wherein said second oligomeric compound comprises a sense oligonucleotide.

45. The composition of claim 43 wherein said second oligomeric compound comprises an oligonucleotide having a plurality of ribose nucleotide units.

46. The composition of claim 43 wherein said first oligomeric compound comprises said at least one conjugate moiety.

47. The composition of claim 43 wherein said second oligomeric compound comprises said at least one conjugate moiety.

48. The composition of claim 43 wherein said first and second oligomeric compounds each comprises at least one conjugate moiety.

49. The composition of claim 43 wherein said second oligomeric compound comprises at least one conjugate moiety and said first oligomeric compound comprises no conjugate moiety.

50. The composition of claim 38 wherein said at least one conjugate moiety is attached to an internal residue of said first or second oligomeric compounds.

51. The composition of claim 38 wherein said at least one conjugate moiety is attached to a terminal residue of said first or second oligomeric compound.

52. The composition of claim 51 wherein said terminal residue is at the 5' end of said first or second oligomeric compound.

53. The composition of claim 38 wherein said conjugate moiety is attached to a heterocyclic base moiety of said first or second oligomeric compound.

54. The composition of claim 38 wherein said at least one conjugate moiety is attached to a monomeric subunit of said first or second oligomeric compound.

55. The composition of claim 38 wherein said at least one conjugate moiety is attached to a monomeric subunit linkage of said first or second oligomeric compounds.

56. The composition of claim 38 wherein said at least one conjugate moiety is attached to said first or second oligomeric compounds through a linker.

57. The composition of claim 38 wherein said at least one conjugate moiety is a lipophilic moiety, vitamin, polymer, peptide, protein, nucleic acid, small molecule, oligosaccharide, carbohydrate cluster, intercalator, minor groove binder, cleaving agent, or cross-linking agent.

58. The composition of claim 38 wherein said at least one conjugate moiety is a steroid.

59. The composition of claim 38 wherein said at least one conjugate moiety is cholesterol or a cholesterol derivative.

60. The composition of claim 38 wherein said at least one conjugate moiety binds to low-density lipoprotein.

61. The composition of claim 38 wherein said at least one conjugate moiety is folate or folate derivative.

62. The composition of claim 38 wherein said at least one conjugate moiety is a water-soluble polymer.

63. The composition of claim 38 wherein said at least one conjugate moiety comprises polyethylene glycol or copolymer thereof.

64. The composition of claim 38 wherein said at least one conjugate moiety comprises a fusogenic peptide or delivery peptide.

65. The composition of claim 38 wherein said at least one conjugate moiety comprises a nuclear export signal.

66. The composition of claim 38 wherein said at least one conjugate moiety comprises a nucleic acid.

67. The composition of claim 38 wherein said at least one conjugate moiety comprises a drug.

68. The composition of claim 38 wherein said at least one conjugate moiety binds to human **serum albumin**.

69. The composition of claim 38 wherein said at least one conjugate moiety comprises a reporter group.

70. An oligomeric compound comprising a first region and a second region, wherein said first region is capable of hybridizing with said second region, wherein a portion of said oligomeric compound is capable of hybridizing to a target nucleic acid, and wherein said oligomeric compound further comprises at least one conjugate moiety.

71. The oligomeric compound of claim 70 wherein each of said first and said second regions comprise at least 10 nucleotides.

72. The oligomeric compound of claim 70 wherein said first region in a 5' to 3' direction is complementary to said second region in a 3' to 5' direction.

73. The oligomeric compound of claim 70 wherein said oligomeric compound comprises a hairpin structure.

74. The oligomeric compound of claim 70 further comprising a third region located between said first region and said second region.

75. The oligomeric compound of claim 74 wherein said third region comprises at least two oligomeric residues.

76. The oligomeric compound of claim 74 wherein said oligomeric compound is RNA.

77. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to an internal residue of said oligomeric compound.

78. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to a terminal residue of said oligomeric compound.

79. The oligomeric compound of claim 78 wherein said terminal residue is at the 5' end of said oligomeric compound.

80. The oligomeric compound of claim 78 wherein said terminal residue is at the 3' end of said oligomeric compound.

81. The oligomeric compound of claim 70 wherein said conjugate moiety is attached to a heterocyclic base moiety of said oligomeric compound.

82. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to a monomeric subunit of said oligomeric compound.

83. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to an monomeric subunit linkage of said oligomeric compound.

84. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is attached to said oligomeric compound trough a linker.

85. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is a lipophilic moiety, vitamin, polymer, peptide, protein, nucleic acid, small molecule, oligosaccharide, carbohydrate cluster, intercalator, minor groove binder, cleaving agent, or cross-linking agent.

86. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is a steroid.

87. The oligomeric compound of claim 70 wherein said at least one conjugate moiety is cholesterol or a cholesterol derivative.

88. The oligomeric compound of claim 70 wherein said at least one conjugate moiety binds to low-density lipoprotein.

89. The composition of claim 70 wherein said at least one conjugate moiety is folate and folate derivatives.

90. The composition of claim 70 wherein said at least one conjugate moiety is a water-soluble polymer.

91. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises polyethylene glycol or copolymer thereof.

92. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises a fusogenic peptide or delivery peptide.

93. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises a nuclear export signal.

94. The oligomeric compound of claim 70 wherein said at least one

conjugate moiety comprises a nucleic acid.

95. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises a drug.

96. The oligomeric compound of claim 70 wherein said at least one conjugate moiety binds to human **serum albumin**.

97. The oligomeric compound of claim 70 wherein said at least one conjugate moiety comprises a reporter group.

98. The oligomeric compound of claim 70 wherein said at least one conjugate moiety localizes said oligomeric compound to the cytoplasm of a cell.

99. The oligomeric compound of claim 70 wherein said at least one conjugate moiety enhances the pharmacokinetic or pharmacodynamic properties of said oligomeric compound.

100. The oligomeric compound of claim 70 wherein said at least one conjugate moiety improves cellular uptake of said oligomeric compound.

101. A pharmaceutical composition comprising the composition of claim 1 and a pharmaceutically acceptable carrier.

102. A pharmaceutical composition comprising the composition of claim 38 and a pharmaceutically acceptable carrier.

103. A pharmaceutical composition comprising the oligomeric compound of claim 70 and a pharmaceutically acceptable carrier.

104. A method of modulating the expression of a target nucleic acid in a cell comprising contacting said cell with a composition of claim 1.

105. A method of modulating the expression of a target nucleic acid in a cell comprising contacting said cell with a composition of claim 38.

106. A method of modulating the expression of a target nucleic acid in a cell comprising contacting said cell with an oligomeric compound of claim 70.

107. A method of treating or preventing a disease or disorder associated with a target nucleic acid comprising administering to an animal having or predisposed to said disease or disorder a therapeutically effective amount of a composition of claim 1.

108. A method of treating or preventing a disease or disorder associated with a target nucleic acid comprising administering to an animal having or predisposed to said disease or disorder a therapeutically effective amount of a composition of claim 38.

109. A method of treating or preventing a disease or disorder associated with a target nucleic acid comprising administering to an animal having or predisposed to said disease or disorder a therapeutically effective amount of an oligomeric compound of claim 70.

L13 ANSWER 3 OF 11 USPATFULL on STN
2005:56706 Genetically altered antibody-producing cell lines with improved antibody characteristics.
Grasso, Luigi, Bala Cynwyd, PA, UNITED STATES
Nicolaides, Nicholas C., Boothwyn, PA, UNITED STATES
Sass, Philip M., Audubon, PA, UNITED STATES
US 2005048621 A1 20050303
APPLICATION: US 2004-933034 A1 20040902 (10)
PRIORITY: US 2003-500071P 20030903 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Dominant negative alleles of human mismatch repair genes can be used to generate hypermutable cells and organisms. Cells may be selected for expression of activation-induced cytidine deaminase (AID), stimulated to produce AID, or manipulated to express AID for further enhancement of hypermutability. These methods are useful for generating genetic diversity within immunoglobulin genes directed against an antigen of interest to produce altered antibodies with enhanced biochemical activity. Moreover, these methods are useful for generating antibody-producing cells with increased level of antibody production.
CLM What is claimed is:
1. A method for generating a hypermutable antibody-producing cell in

vitro comprising: introducing into an antibody-producing cell a polynucleotide comprising a dominant negative allele of a mismatch repair gene; and stimulating expression of activation-induced cytidine deaminase; thereby generating a hypermutable antibody-producing cell.

2. The method of claim 1 wherein said mismatch repair gene encodes a truncated form of a PMS2.

3. The method of claim 2 wherein said PMS2 is a mammalian PMS2.

4. The method of claim 2 wherein said PMS2 is a rodent PMS2.

5. The method of claim 2 wherein said PMS2 is a human PMS2.

6. The method of claim 2 wherein said PMS2 is a plant PMS2.

7. The method of claim 2 wherein said truncated form of a PMS2 consists of an amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:33, and SEQ ID NO:37.

8. The method of claim 1 wherein said expression of activation-induced cytidine deaminase is stimulated by incubating said cells with at least one activating cytokine.

9. The method of claim 8 wherein said activating cytokine is selected from the group consisting of LPS, CD40L, TGFβ, and IL-4.

10. An isolated, hypermutable, antibody-producing cell produced by the method of claim 1.

11. A culture of the cells of claim 10.

12. The method of claim 1 further comprising the step of inactivating the dominant negative allele of said mismatch repair gene of said polynucleotide, thereby producing a genetically stable antibody-producing cell.

13. An isolated, genetically stable, antibody-producing cell produced by the method of claim 12 wherein said antibody-producing cell produces an antibody with higher affinity than that produced prior to the introduction of said polynucleotide comprising said dominant negative allele of a mismatch repair gene.

14. A culture of cells of claim 13.

15. An isolated, genetically stable, antibody-producing cell produced by the method of claim 12 wherein said antibody-producing cell produces a higher titer of antibody than that produced prior to the introduction of said polynucleotide comprising said dominant negative allele of a mismatch repair gene.

16. A homogeneous culture of genetically stable cells of claim 13.

17. A method for producing hybridoma cells producing antibodies from in vitro immunized immunoglobulin-producing cells comprising: (a) combining cells capable of producing immunoglobulins with an immunogenic antigen in vitro; (b) fusing said cells with myeloma cells to form parental hybridoma cells; (c) selecting for cells that express activation induced cytidine deaminase; (d) incubating said parental hybridoma cells to allow for mutagenesis, thereby forming hypermutated hybridoma cells; (e) selecting hypermutated hybridoma cells that produce antibodies that specifically bind antigen, thereby producing hybridoma cells producing antibodies from in vitro immunized immunoglobulin-producing cells.

18. The method of claim 17 wherein said activation-induced cytidine deaminase is a mammalian activation-induced cytidine deaminase.

19. The method of claim 18 wherein said activation-induced cytidine deaminase is a human activation-induced cytidine deaminase.

20. The method of claim 17 wherein said activation-induced cytidine deaminase is a mouse activation-induced cytidine deaminase.

21. The method of claim 17 wherein said parental hybridoma cell expresses a dominant negative allele of a mismatch repair gene.

22. The method of claim 17 wherein said mismatch repair gene is selected from the group consisting of PMS1, PMS2, MLH1, MLH3, MSH2, MSH3, MSH4,

MSH5, MSH6, PMSR6 and PMSL9.

23. The method of claim 22 wherein said mismatch repair gene encodes a truncated form of a PMS2 protein.

24. The method of claim 23 wherein said truncated form of a PMS2 protein comprises PMS2-134.

25. The method of claim 23 wherein said PMS2 is a plant PMS2.

26. The method of claim 23 wherein said PMS2 is a mammalian PMS2.

27. The method of claim 26 wherein said PMS2 is a human PMS2.

28. The method of claim 17 wherein said parental hybridoma cells are transfected with a dominant negative allele of a mismatch repair gene.

29. The method of claim 17 wherein said myeloma cells comprise a dominant negative allele of a mismatch repair gene.

30. The method of claim 29 wherein said myeloma cells are transfected with a dominant negative allele of a mismatch repair gene.

31. The method of claim 17 wherein said antibody-producing cells comprise a dominant negative allele of a mismatch repair gene.

32. The method of claim 31 wherein said antibody-producing cells are transfected with a dominant negative allele of a mismatch repair gene.

33. The method of claim 17 wherein said parental hybridoma cells are transfected with a dominant negative allele of a mismatch repair gene.

34. The method of claim 17 further comprising the step of incubating said parental hybridoma cells with a chemical inhibitor of mismatch repair.

35. A method for generating a hypermutable antibody-producing cell in vitro comprising: introducing into an antibody-producing cell a polynucleotide comprising a dominant negative allele of a mismatch repair gene; culturing said antibody-producing cell to yield a population of antibody-producing cells; and selecting an antibody-producing cell from said population that expresses activation-induced cytidine deaminase; thereby generating a hypermutable antibody-producing cell.

36. The method of claim 35 wherein said antibody producing cell is a hybridoma cell.

37. The method of claim 35 wherein said antibody-producing cell is a mammalian expression cell transfected with polynucleotides encoding immunoglobulin heavy and light chains.

38. A method for generating a hypermutable antibody-producing cell in vitro comprising: stimulating expression of activation-induced cytidine deaminase in a mismatch repair deficient antibody-producing cell, thereby generating a hypermutable antibody-producing cell.

39. The method of claim 38 wherein said antibody-producing cells are stimulated with at least one activating cytokine selected from the group consisting of CD40L, TGFβ, IL-IL-4, and LPS.

40. A method for generating a hypermutable antibody-producing cell in vitro comprising: stimulating expression of activation-induced cytidine deaminase in an antibody-producing cell, thereby generating a hypermutable antibody-producing cell.

41. The method of claim 40 wherein said antibody-producing cells are stimulated with at least one activating cytokine selected from the group consisting of CD40L, TGFβ, IL-IL-4, and LPS.

42. A method for generating a hypermutable antibody-producing cell in vitro comprising: culturing antibody producing cells and selecting an antibody-producing cell that expresses activation-induced cytidine deaminase, thereby generating a hypermutable antibody-producing cell.

43. A method for generating a hypermutable antibody-producing cell in vitro comprising: culturing antibody producing cells that are deficient in mismatch repair and selecting an antibody-producing cell that expresses activation-induced cytidine deaminase, thereby generating a

hypermutable antibody-producing cell.

L13 ANSWER 4 OF 11 USPATFULL on STN
2004:138961 DCR-5 bone affecting ligand.
Economides, Aris N., Tarrytown, NY, UNITED STATES
Stahl, Neil, Carmel, NY, UNITED STATES
US 2004106134 A1 20040603
APPLICATION: US 2003-662756 A1 20030915 (10)
PRIORITY: US 1998-97296P 19980820 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB DCR5, a protein related to DAN (Differential-screening-selected gene Aberrative in Neuroblastoma) and related nucleic acids are provided. Included are natural DCR5 homologs from several species and proteins comprising a DCR5 domain having specific activity, particularly the ability to antagonize a bone morphogenetic protein. The proteins may be produced recombinantly from transformed host cells with the subject nucleic acids. Also provided are isolated hybridization probes and primers capable of specifically hybridizing with the disclosed genes, specific binding agents and methods of making and using the subject compositions.
CLM What is claimed is:
1. Isolated human DCR5 protein.

2. The isolated human DCR5 protein of claim 1, having the amino acid sequence as set forth in SEQ ID NO: 12.

3. Protein domains having DCR5-specific activity, such domains comprising at least 6 and preferably at least 8 consecutive residues of human DCR5 protein of claim 1.

4. An antibody which specifically binds the isolated human DCR5 protein of claim 1.

5. The antibody of claim 4, which is a monoclonal antibody.

6. A composition comprising the human DCR5 protein of claim 1, in a pharmaceutically acceptable carrier.

7. A composition comprising the antibody of claim 4, in a pharmaceutically acceptable carrier.

8. A human DCR5 protein produced by the method of: a) constructing a vector comprising a nucleic acid molecule containing a nucleotide sequence encoding human DCR5 protein as set forth in SEQ ID NO: 12, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell; b) introducing the vector of (a) into a host cell; c) growing the host cell of (b) under conditions which permit the production of human DCR5 protein; and d) recovering the human DCR5 protein so produced.

9. The protein of claim 7, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

10. The human DCR5 protein of claim 1, fused to an immunoglobulin constant region.

11. The human DCR5 protein of claim 10, wherein the immunoglobulin constant region is the Fc portion of human IgG1.

12. A method of regulating cartilage and bone growth in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the human DCR5 protein of claim 1 such that cartilage and bone growth are regulated.

13. A method of regulating cartilage and bone growth in a subject in need thereof comprising administering to the subject a therapeutically effective amount of the antibody of claim 4 such that cartilage and bone growth are regulated.

L13 ANSWER 5 OF 11 USPATFULL on STN
2004:51494 Inhibition of complement activation and complement modulation by use of modified oligonucleotides.
Henry, Scott, Cardiff, CA, UNITED STATES
US 2004038925 A1 20040226
APPLICATION: US 2003-636452 A1 20030807 (10)
DOCUMENT TYPE: Utility; APPLICATION.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Methods for inhibiting complement activation using antisense oligonucleotides, preferably modified oligonucleotides. These compounds may be used therapeutically to treat undesirable complement-mediated events such as inflammation.

CLM What is claimed is:

1. A method for inhibiting complement activation in a human cell, tissue or bodily fluid comprising contacting said cell, tissue or bodily fluid with an oligonucleotide comprising at least one 2' sugar modification.

2. The method of claim 1, wherein said 2' sugar modification is a 2'-methoxyethoxy modification.

3. The method of claim 1, wherein said oligonucleotide has at least one modified internucleotide linkage.

4. The method of claim 3, wherein said modified linkage is a phosphorothioate.

5. The method of claim 1, wherein said oligonucleotide consists of phosphorothioate linkages.

6. The method of claim 1, wherein said oligonucleotide is an antisense oligonucleotide.

7. The method of claim 1, wherein the concentration of said oligonucleotide is at least about 50 micrograms/ml.

8. The method of claim 7, wherein the concentration of said oligonucleotide is between about 50 micrograms/ml and 250 micrograms/ml.

9. A composition comprising an oligonucleotide and a complement activation inhibitory molecule, wherein said oligonucleotide comprises at least one phosphorothioate modification and at least one 2'-methoxyethoxy modification.

10. The composition of claim 9 wherein said complement activation inhibitory molecule is Factor H.

L13 ANSWER 6 OF 11 USPATFULL on STN

2003:321429 DCR5, a BMP-binding protein, and applications thereof.

Economides, Aris N., Tarrytown, NY, United States
Stahl, Neil, Carmel, NY, United States
Regeneron Pharmaceuticals, Inc., Tarrytown, NY, United States (U.S. corporation)

US 6660499 B1 20031209
WO 2000011163 20000302
APPLICATION: US 2001-762960 20010214 (9)
WO 1999-US17979 19990812
PRIORITY: US 1998-97296P 19980820 (60)
DOCUMENT TYPE: Utility; GRANTED.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB DCR5, a protein related to DAN (Differential-screening-selected gene Aberrative in Neuroblastoma) and related nucleic acids are provided. Included are natural DCR5 homologs from several species and proteins comprising a DCR5 domain having specific activity, particularly the ability to antagonize a bone morphogenetic protein. The proteins may be produced recombinantly from transformed host cells with the subject nucleic acids. Also provided are isolated hybridization probes and primers capable of specifically hybridizing with the disclosed genes, specific binding agents and methods of making and using the subject compositions.

CLM What is claimed is:

1. An isolated nucleic acid molecule encoding human DCR5 as set forth in SEQ ID NO:12.

2. An isolated nucleic acid molecule according to claim 1, having a sequence selected from the group consisting of: (a) the nucleotide sequence comprising the coding region of the human DCR5 as set forth in SEQ ID NO. 11; and (b) a nucleotide sequence which, as a result of the degeneracy of the genetic code, differs from the nucleic acid of (a) and which encodes human DCR5.

3. A vector which comprises a nucleic acid molecule of claim 1 or 2.

4. A vector according to claim 3, wherein the nucleic acid molecule is operatively linked to an expression control sequence capable of directing its expression in a host cell.

5. A vector according to claim 3 which is a plasmid.

6. A host-vector system for the production of human DCR5 which comprises a vector of claim 3 in a host cell.

7. A host-vector system according to claim 6, wherein the host cell is a bacterial, yeast, insect or mammalian cell.

8. A method of producing human DCR5 which comprises growing cells of a host-vector system of claim 6 under conditions permitting production of the human DCR5, and recovering the human DCR5 so produced.

9. An isolated nucleic acid consisting of a nucleotide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 9.

L13 ANSWER 7 OF 11 USPATFULL on STN
2003:146820 Tyrosine kinase inhibitors.
Bilodeau, Mark T., Lansdale, PA, UNITED STATES
Hartman, George D., Lansdale, PA, UNITED STATES
US 2003100567 A1 20030529
APPLICATION: US 2002-174774 A1 20020619 (10)
PRIORITY: US 2001-300245P 20010622 (60)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to compounds which inhibit, regulate and/or modulate tyrosine kinase signal transduction, compositions which contain these compounds, and methods of using them to treat tyrosine kinase-dependent diseases and conditions, such as angiogenesis, cancer, tumor growth, atherosclerosis, age related macular degeneration, diabetic retinopathy, inflammatory diseases, and the like in mammals.

CLM What is claimed is:

1. A compound of Formula I ##STR33## or a pharmaceutically acceptable salt or stereoisomer thereof, wherein n is 0 or 1; X is C--H or N, provided X is C--H if n=1 and R^1 is SO_2--(C_{1-C6} alkyl) and provided that X is C--H if R^1 is NH(C.dbd.O)NR^{3H}; R^1 is 1) SO_2--(C_{1-C6} alkyl), 2) (C.dbd.O)NR^{3H}, or 3) NH(C.dbd.O)NR^{3H}; R^2 is 1) H, 2) OH, 3) OC_{1-C6} alkyl, 4) C_{1-C6} alkyl, or 5) halo; and R^3 is C_{1-C6} alkyl.

2. The compound of claim 1, or a pharmaceutically acceptable salt thereof, wherein n is 1; X is N; and R^1 is (C.dbd.O)NR^{3H}.

3. A compound selected from: 4-[2-(5-cyano-thiazol-2-ylamino)-pyridin-4-ylmethyl]-piperazine-1-carboxylic acid methylamide; 2-[(4-{[4-(methylsulfonyl)piperidin-1-yl]methyl}pyridin-2-yl)amino]-1,3-thiazole-5-carbonitrile; N-[(3R)-1-({2-[(5-cyano-1,3-thiazol-2-yl)amino]pyridin-4-yl}methyl)pyrrolidin-3-yl]-N'-methylurea; 2-{[4-({[(3R)-5-oxopyrrolidin-3-yl]amino}methyl)pyridin-2-yl]amino}-1,3-thiazole-5-carbonitrile; 4-[2-(5-cyano-thiazol-2-ylamino)-5-methyl-pyridin-4-ylmethyl]-piperazine-1-carboxylic acid methylamide; 4-[2-(5-cyano-thiazol-2-ylamino)-3-methyl-pyridin-4-ylmethyl]-piperazine-1-carboxylic acid methylamide; 4-({2-chloro-6-[(5-cyano-1,3-thiazol-2-yl)amino]pyridin-4-yl}methyl)-N-methylpiperazine-1-carboxamide; 4-({2-[(5-cyano-1,3-thiazol-2-yl)amino]-6-ethylpyridin-4-yl}methyl)-N-methylpiperazine-1-carboxamide; and 2-({4-[(4-acetylpiperazin-1-yl)methyl]-6-methylpyridin-2-yl} amino)-1,3-thiazole-5-carbonitrile; or a pharmaceutically acceptable salt or stereoisomer thereof.

4. A compound which is ##STR34## 4-[2-(5-cyano-thiazol-2-ylamino)-pyridin-4-ylmethyl]-piperazine-1-carboxylic acid methylamide, or a pharmaceutically acceptable salt thereof.

5. A compound which is ##STR35## 2-[(4-{[4-(methylsulfonyl)piperidin-1-yl]methyl}pyridin-2-yl)amino]-1,3-thiazole-5-carbonitrile, or a pharmaceutically acceptable salt thereof.

6. A compound which is ##STR36## N-[(3R)-1-({2-[(5-cyano-1,3-thiazol-2-yl)amino]pyridin-4-yl}methyl)pyrrolidin-3-yl]-N'-methylurea, or a pharmaceutically acceptable salt or stereoisomer thereof.

7. A compound which is ##STR37## 2-{[4-({[(3R)-5-oxopyrrolidin-3-yl]amino} methyl)pyridin-2-yl]amino}-1,3-thiazole-5-carbonitrile, or a pharmaceutiaclly acceptable salt or stereoisomer thereof.

8. A compound which is ##STR38## 4-[2-(5-cyano-thiazol-2-ylamino)-3-

methyl-pyridin-4-ylmethyl]-piperazine-1-carboxylic acid methylamide, or a pharmaceutically accepatble salt thereof.

9. A pharmaceutical composition which is comprised of a compound in accordance with claim 1 and a pharmaceutically acceptable carrier.

10. A method of treating or preventing cancer in a mammal in need of such treatment which is comprised of administering to said mammal a therapeutically effective amount of a compound of claim 1.

11. A method of treating cancer or preventing cancer in accordance with claim 10 wherein the cancer is selected from cancers of the brain, genitourinary tract, lymphatic system, stomach, larynx and lung.

12. A method of treating or preventing cancer in accordance with claim 10 wherein the cancer is selected from histiocytic lymphoma, lung adenocarcinoma, small cell lung cancers, pancreatic cancer, glioblastomas and breast carcinoma.

13. A method of treating or preventing a disease in which angiogenesis is implicated, which is comprised of administering to a mammal in need of such treatment a therapeutically effective amount of a compound of claim 1.

14. A method in accordance with claim 13 wherein the disease is an ocular disease.

15. A method of treating or preventing retinal vascularization which is comprised of administering to a mammal in need of such treatment a therapeutically effective amount of compound of claim 1.

16. A method of treating or preventing diabetic retinopathy which is comprised of administering to a mammal in need of such treatment a therapeutically effective amount of compound of claim 1.

17. A method of treating or preventing age-related macular degeneration which is comprised of administering to a mammal in need of such treatment a therapeutically effective amount of a compound of claim 1.

18. A method of treating or preventing inflammatory diseases which comprises administering to a mammal in need of such treatment a therapeutically effective amount of a compound of claim 1.

19. A method according to claim 18 wherein the inflammatory disease is selected from rheumatoid arthritis, psoriasis, contact dermatitis and delayed hypersensitivity reactions.

20. A method of treating or preventing a tyrosine kinase-dependent disease or condition which comprises administering a therapeutically effective amount of a compound of claim 1.

21. A pharmaceutical composition made by combining the compound of claim 1 and a pharmaceutically acceptable carrier.

22. A process for making a pharmaceutical composition which comprises combining a compound of claim 1 with a pharmaceutically acceptable carrier.

23. A method of treating or preventing bone associated pathologies selected from osteosarcoma, osteoarthritis, and rickets which comprises administering a therapeutically effective amount of a compound of claim 1.

24. The composition of claim 9 further comprising a second compound selected from: 1) an estrogen receptor modulator, 2) an androgen receptor modulator, 3) retinoid receptor modulator, 4) a cytotoxic agent, 5) an antiproliferative agent, 6) a prenyl-protein transferase inhibitor, 7) an HMG-CoA reductase inhibitor, 8) an HIV protease inhibitor, 9) a reverse transcriptase inhibitor, and 10) another angiogenesis inhibitor.

25. The composition of claim 24, wherein the second compound is another angiogenesis inhibitor selected from the group consisting of a tyrosine kinase inhibitor, an inhibitor of epidermal-derived growth factor, an inhibitor of fibroblast-derived growth factor, an inhibitor of platelet derived growth factor, an MMP inhibitor, an integrin blocker, interferon-α, interleukin-12, pentosan polysulfate, a cyclooxygenase inhibitor, carboxyamidotriazole, combretastatin A-4, squalamine, 6-O-chloroacetyl-carbonyl)-fumagillol, thalidomide,

angiostatin, troponin-1, and an antibody to VEGF.

26. The composition of claim 24, wherein the second compound is an estrogen receptor modulator selected from tamoxifen and raloxifene.

27. A method of treating cancer which comprises administering a therapeutically effective amount of a compound of claim 1 in combination with radiation therapy.

28. A method of treating or preventing cancer which comprises administering a therapeutically effective amount of a compound of claim 1 in combination with a compound selected from: 1) an estrogen receptor modulator, 2) an androgen receptor modulator, 3) retinoid receptor modulator, 4) a cytotoxic agent, 5) an antiproliferative agent, 6) a prenyl-protein transferase inhibitor, 7) an HMG-CoA reductase inhibitor, 8) an HIV protease inhibitor, 9) a reverse transcriptase inhibitor, and 10) another angiogenesis inhibitor.

29. A method of treating cancer which comprises administering a therapeutically effective amount of a compound of claim 1 in combination with radiation therapy and a compound selected from: 1) an estrogen receptor modulator, 2) an androgen receptor modulator, 3) retinoid receptor modulator, 4) a cytotoxic agent, 5) an antiproliferative agent, 6) a prenyl-protein transferase inhibitor, 7) an HMG-CoA reductase inhibitor, 8) an HIV protease inhibitor, 9) a reverse transcriptase inhibitor, and 10) another angiogenesis inhibitor.

30. A method of treating or preventing cancer which comprises administering a therapeutically effective amount of a compound of claim 1 and paclitaxel or trastuzumab.

31. A method of treating or preventing cancer which comprises administering a therapeutically effective amount of a compound of claim 1 and a GPIIb/IIIa antagonist.

32. The method of claim 31 wherein the GPIIb/IIIa antagonist is tirofiban.

33. A method of reducing or preventing tissue damage following a cerebral ischemic event which comprises administering a therapeutically effective amount of a compound of claim 1.

34. A method of treating or preventing cancer which comprises administering a therapeutically effective amount of a compound of claim 1 in combination with a COX-2 inhibitor.

35. A method of treating or preventing preeclampsia which comprises administering a therapeutically effective amount of a compound of claim 1.

36. A method of preventing tumor cell metastasis which comprises adminstering a therapeutically effective amount of a compound of claim 1.

L13 ANSWER 8 OF 11 USPATFULL on STN
2002:157622 Use of oligonucleotides for inhibition of complement activation.
Henry, Scott, Cardiff, CA, UNITED STATES
US 2002082227 A1 20020627
APPLICATION: US 2001-794824 A1 20010227 (9)
DOCUMENT TYPE: Utility; APPLICATION.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods for inhibiting complement activation using antisense oligonucleotides, preferably modified oligonucleotides. These compounds may be used therapeutically to treat undesirable complement-mediated events such as inflammation.
CLM What is claimed is:
1. A method for inhibiting complement activation in a human cell, tissue or bodily fluid comprising administering an oligonucleotide to said cell, tissue or bodily fluid.

2. The method of claim 1, wherein said oligonucleotide comprises one or more modifications.

3. The method of claim 2, wherein said modification is an internucleoside linkage.

4. The method of claim 3, wherein said linkage is a phosphorothioate linkage.

5. The method of claim 1, wherein said modification is a modification at the 2'-position of a sugar.

6. The method of claim 5, wherein said modification is a 2'-O-methoxyethyl modification.

7. The method of claim 4, wherein said oligonucleotide consists of phosphorothioate linkages.

8. The method of claim 1, wherein said oligonucleotide is selected from the group consisting of ISIS 13650, ISIS 15839, ISIS 12854 and ISIS 14725.

9. The method of claim 1, wherein said oligonucleotide is selected from the group consisting of ISIS 5132 and ISIS 2302.

10. The method of claim 1, wherein the concentration of said oligonucleotide is at least about 50 μg/ml.

11. The method of claim 10, wherein the concentration of said oligonucleotide is between about 50 μg/ml and about 250 μg/ml.

12. A composition comprising an oligonucleotide and a complement activation inhibitory molecule, wherein said oligonucleotide comprises one or more phosphorothioate modifications and one or more 2'-methoxyethoxy modifications.

13. The composition of claim 12 wherein said complement activation inhibitory molecule is Factor H.

L13 ANSWER 9 OF 11 USPATFULL on STN
2001:71531 Inhibition of complement activation and complement modulation by use of modified oligonucleotides.
Henry, Scott, Cardiff, CA, United States
Isis Pharmaceuticals, Inc., Carlsbad, CA, United States (U.S. corporation)
US 6232296 B1 20010515
APPLICATION: US 1999-409816 19990930 (9)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Oligomeric compounds are described wherein said compounds comprise modified oligonucleotides (P.dbd.S) which modulate complement activity. Methods and processes for the uses of such oligomeric compounds are also described. The oligomeric compounds may be used therapeutically to modulate complement activity in order to inhibit undesirable complement mediated events, such as for example, to treat inflammation, and/or to activate complement.
CLM What is claimed is:
1. A method for modulating complement activation in a cell, tissue or a bodily fluid comprising independently administering to said cell, tissue or bodily fluid a first concentration and a second, independent concentration of an oligonucleotide which comprises one or more phosphorothioate modifications, wherein said oligonucleotide initiates complement activation at said first concentration and inhibits complement activation at said second, independent concentration.

2. The method of claim 1, wherein said first concentration is less than or equal to 80 μg/ml.

3. The method of claim 1, wherein said second concentration is at least 200 μg/ml.

L13 ANSWER 10 OF 11 USPATFULL on STN
92:42869 Oxidation-resistant muteins of Il-2 and other protein.
Koths, Kirston E., El Cerrito, CA, United States
Halenbeck, Robert F., San Rafael, CA, United States
Mark, David F., Plainsboro, NJ, United States
Nitecti, Danutee, Berkeley, CA, United States
Cetus Corporation, Emeryville, CA, United States (U.S. corporation)
US 5116943 19920526
APPLICATION: US 1988-192293 19880510 (7)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A biologically active reference therapeutic protein is protected against oxidation by a method involving substituting a conservative amino acid for each methionyl residue susceptible to chloramine T or peroxide

oxidation, wherein additional, non-susceptible methionyl residues are not so substituted. The oxidation-resistant mutein so produced is preferably a human mutein of interleukin-2 or interferon-β, and the conservative amino acid is most preferably alanine.

CLM What is claimed is:

1. An oxidation-resistant IL-2 mutein having therapeutically useful biological activity, wherein each methionine residue of a reference protein susceptible to chloramine T or peroxide oxidation is replaced by a conservative amino acid, and wherein additional, non-susceptible methionine residues are not so substituted.

2. The oxidation-resistant mutein of claim 1 wherein the conservative amino acid is selected from the group consisting of glycine, alanine, serine, threonine, valine, isoleucine, leucine, asparagine, glutamine, glutamate, tyrosine, and phenylalanine.

3. The oxidation-resistant mutein of claim 2 wherein the reference protein is human.

4. The oxidation-resistant mutein of claim 2 wherein the conservative amino acid is selected from the group consisting of alanine, serine, leucine, isoleucine, glutamate and valine.

5. The oxidation-resistant mutein of claim 4 selected from the group consisting ala_{104} ser_{125} IL-2, ala_{104} ala_{125} IL-2, val_{104} ser_{125} IL-2, val_{104} IL-2, val_{104} ala_{125} IL-2, des-ala_1 ala_{104} ser_{125} IL-2, des-ala_1 ala_{104} IL-2, des-ala_1 ala_{104} ala_{125} IL-2, des-ala_1 val_{104} ser_{125} IL-2, des-ala_1 val_{104} IL_2, des-ala_1 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 ala_{104} IL-2, des-ala_1 des-pro_2 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 val_{104} IL-2, des-ala_1 des-pro_2 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 val_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 val_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 val_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} IL-2, and des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} ser_{125} IL-2.

6. The oxidation-resistant mutein of claim 5 selected from the group consisting of ala_{104} ser_{125} IL-2, ala_{104} IL_2, ala_{104} ala_{125} IL-2, des-ala_1 ala_{104} IL-2, des-ala_1 ala_{104} ser_{125} IL-2, or des-ala_1 ala_{104} ala_{125} IL-2.

7. A human IL-2 oxidation-resistant mutein having a conservative amino acid substitution at position 104, wherein the reference IL-2 protein

contains a methionine at position 104, and wherein said mutein exhibits the biological activity of human IL-2.

8. The mutein of claim 7 wherein the conservative amino acid is selected from the group consisting of glycine, alanine, serine, threonine, valine, isoleucine, leucine, asparagine, glutamine, glutamate, tyrosine, and phenylalanine.

9. The mutein of claim 8 wherein the amino acid is selected from the group consisting of alanine, serine, leucine, glutamate, and valine.

10. The mutein of claim 9 wherein the protein is unglycosylated and the amino acid is serine or alanine.

11. A therapeutic formulation comprising an effective amount of the mutein of claim 7 and an inert, non-allergenic, pharmaceutically compatible carrier.

12. The formulation of claim 11 wherein the mutein is an ala_{104} IL-2 mutein, and the carrier is selected from the group consisting of distilled water, physiological saline, Ringer's solution, and Hank's solution.

13. The formulation of claim 12, further comprising non-toxic stabilizers and solubilizers.

L13 ANSWER 11 OF 11 USPATFULL on STN
88:39172 Oxidation-resistant muteins.
Koths, Kirston E., El Cerrito, CA, United States
Halenbeck, Robert F., San Rafael, CA, United States
Innis, Michael A., Oakland, CA, United States
Wang, Alice M., Walnut Creek, CA, United States
Shaked, Ze'Ev, Berkeley, CA, United States
Cetus Corporation, Emeryville, CA, United States (U.S. corporation)
US 4752585 19880621
APPLICATION: US 1986-893186 19860805 (6)
DOCUMENT TYPE: Utility; Granted.
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A biologically active reference therapeutic protein is protected against oxidation by a method involving substituting a conservative amino acid for each methionyl residue susceptible to chloramine T or peroxide oxidation, wherein additional, non-susceptible methionyl residues are not so substituted. The oxidation-resistant mutein so produced is preferably a human mutein of interleukin-2 or interferon-β, and the conservative amino acid is most preferably alanine.

CLM What is claimed:

1. A recombinant DNA sequence that encodes an interleukin-2 (IL-2) mutein having therapeutically useful biological activity in comparison with a reference protein, wherein said reference protein is either mature native recombinant IL-2 having the primary amino acid sequence shown in FIG. 1 or the des ala_1 ser_{125} IL-2 mutein thereof, and facilitating the production of a less heterogeneous protein preparation, as determined by reverse phase high performance liquid chromatography analysis, in comparison with said reference protein, wherein said IL-2 mutein is oxidation-resistant where each methionine residue of said reference protein that is susceptible to chloramine T or hydrogen peroxide oxidation is replaced by a conservative amino acid, and having additional, non-susceptible methionine residues not so substituted.

2. The recombinant DNA sequence of claim 1 wherein the reference protein for the mutein is des ala_1 ser_{125} IL-2.

3. A recombinant expression vector which comprises the DNA sequence of claim 2 operably linked to control sequences compatible with a suitable host.

4. A recombinant expression vector which comprises the DNA sequence of claim 1 operably linked to control sequences compatible with a suitable host.

5. The recombinant expression vector of claim 4 wherein the host is yeast.

6. The recombinant expression vector of claim 5 which comprises a gene coding for ala_{104} IL-2 which is designated pPM43 and has ATCC No. 53,356.

7. The recombinant expression vector of clam 4 which is pSY3001 wherein the host is E. coli.

8. Host cells transformed with the expression vector of claim 3.

9. Host cells transformed with the expression vector of claim 4.

10. The recombinant DNA sequence of claim 1 wherein the conservative amino acid sequence is selected from the group consisting of alanine, serine, leucine, isoleucine, glutamate and valine.

11. The recombinant DNA sequence of claim 1, which DNA sequence encodes for an oxidation-resistant mutein selected from the group consisting of; ala_{104} ser_{125} IL-2, ala_{104} IL-2, ala_{104} ala_{125} IL-2, val_{104} ser_{125} IL-2, val_{104} IL-2, val_{104} ala_{125} IL-2, des-ala_1 ala_{104} ser_{125} IL-2, des-ala_1 ala_{104} IL-2, des-ala_1 ala_{104} ala_{125} IL-2, des-ala_1 val_{104} ser_{125} IL-2, des-ala_1 val_{104} IL-2, des-ala_1 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 ala_{104} IL-2, des-ala_1 des-pro_2 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 val_{104} IL-2, des-ala_1 des-pro_2 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 ala_{104} IL-2, des-ala_1 des-pro 2 des-thr_3 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 val_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 val_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 val $_{104}$ IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 val_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} ala_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 ala_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 val_{104} ser_{125} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 val_{104} IL-2, des-ala_1 des-pro_2 des-thr_3 des-ser_4 des-ser_5 des-ser_6 val_{104} ala_{125} IL-2.

12. The recombinant DNA sequence of claim 1 which DNA sequence encodes for an oxidation-resistant mutein selected from the group consisting of; ala_{104} ser_{125} IL-2, ala_{104} IL-2, ala_{104} ala_{125} IL-2, des-ala_1 ala_{104} IL-2, des-ala_1 ala_{104} ser_{125} IL-2, or des-ala_1 ala_{104} ala_{125} IL-2.

```
=> d his

     (FILE 'HOME' ENTERED AT 20:00:42 ON 26 NOV 2005)

     FILE 'MEDLINE' ENTERED AT 20:01:07 ON 26 NOV 2005
L1         68146 S (SERUM ALBUMIN)
L2            74 S L1 AND (CYS-34 OR CYSTEINE 34)
L3            39 S L2 AND PY<2000
L4         15041 S L1 AND PHARMACOLOG?
L5          1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6             2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
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L7            47 S L4 AND (MALEIMID?)
L8            28 S L7 AND PY<2000

     FILE 'USPATFULL' ENTERED AT 20:20:48 ON 26 NOV 2005
L9         59637 S (SERUM ALBUMIN)
L10           80 S L9 AND (CYS-34 OR CYSTEINE-34)
L11           13 S L10 AND AY<2000
L12           13 S L9 AND (ENHANCED PHARMACOKINETIC)
L13           11 S L12 NOT L10

=> file medline
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    84.52     112.35

FILE 'MEDLINE' ENTERED AT 20:27:40 ON 26 NOV 2005

 FILE LAST UPDATED: 26 NOV 2005 (20051126/UP).  FILE COVERS 1950 TO DATE.

 On December 19, 2004, the 2005 MeSH terms were loaded.

 The MEDLINE reload for 2005 is now available.  For details enter HELP
 RLOAD at an arrow promt (=>).  See also:

    http://www.nlm.nih.gov/mesh/
    http://www.nlm.nih.gov/pubs/techbull/nd04/nd04_mesh.html

 OLDMEDLINE now back to 1950.

 MEDLINE thesauri in the /CN, /CT, and /MN fields incorporate the
 MeSH 2005 vocabulary.

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

=> s l1 and (enhanced pharmacokinetic)
        258925 ENHANCED
         40523 PHARMACOKINETIC
            11 ENHANCED PHARMACOKINETIC
                 (ENHANCED(W)PHARMACOKINETIC)
L14          0 L1 AND (ENHANCED PHARMACOKINETIC)

=> s l1 and pharmacokinetic
         40523 PHARMACOKINETIC
L15        434 L1 AND PHARMACOKINETIC

=> s l15 and (enhanced or increased or greater)
        258925 ENHANCED
       1214534 INCREASED
        503507 GREATER
L16        144 L15 AND (ENHANCED OR INCREASED OR GREATER)

=> sl16 and py<2000
SL16 IS NOT A RECOGNIZED COMMAND
The previous command name entered was not recognized by the system.
For a list of commands available to you in the current file, enter
"HELP COMMANDS" at an arrow prompt (=>).

=> s l16 and py<2000
      12397709 PY<2000
L17        103 L16 AND PY<2000

=> s l17 and albumin/ab
         67629 ALBUMIN/AB
L18         86 L17 AND ALBUMIN/AB

=> s l18 (conjugat?)
MISSING OPERATOR 'L18 (CONJUGAT?'
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l18 and conjugat?
         78444 CONJUGAT?
L19          9 L18 AND CONJUGAT?

=> d l19,cbib,ab,1-9

L19  ANSWER 1 OF 9     MEDLINE on STN
2000163906.    PubMed ID: 10701971.   High sensitivity ELISA determination of
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taxol in various human biological fluids. Svojanovsky S R; Egodage K L; Wu J; Slavik M; Wilson G S. (Department of Chemistry, University of Kansas, Lawrence 66045, USA.) Journal of pharmaceutical and biomedical analysis, **(1999 Jul)** 20 (3) 549-55. Journal code: 8309336. ISSN: 0731-7085. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Taxol (paclitaxel)--the natural product isolated from Pacific yew (Taxus brevifolia)--is a novel agent with high activity in the treatment of patients with several malignant tumors including those resistant to other cytotoxic drugs. The therapeutic index of this promising anticancer drug could be further **increased** by the exploration of its **pharmacokinetic** pharmacodynamic relationship in cancer patients. Since taxol is highly protein bound, a very specific and highly sensitive analytical method is required in order to determine free, protein unbound and biologically active taxol species in human physiological fluids: plasma; plasma ultrafiltrate; and salivary fluids. In order to accomplish this, a new indirect competitive enzyme-linked immunosorbent assay (ELISA), for quantitating such a low bioactive taxol concentration level, has been developed in our laboratories. This method uses taxol competitive inhibition of mouse anti-taxol antibodies binding to the solid phase coated antigen 7-succinyltaxol-bovine **serum albumin**. This indicates recognition of the active taxol in the solution phase, where a diluted horseradish peroxidase labeled goat anti-mouse enzyme **conjugate** is used. While employing this technique, after systematic optimization of the experimental conditions, we are able to detect the anticipated taxol in plasma ultrafiltrate and salivary fluids at the concentration level of subpicogram per milliliter. The working range of the assay is approximately five orders in magnitude, i.e. from pg ml(-1) to 100 ng ml(-1). The clinical part of this study verified the working range of the ELISA method using samples of physiological fluids from a cancer patient treated with 3 h intravenous (i.v.) infusion of this drug. Our results of taxol determination in plasma, plasma ultrafiltrate and saliva demonstrate the applicability of the newly developed ELISA method for further **pharmacokinetic** studies of free, biologically active taxol species in cancer patients.

L19 ANSWER 2 OF 9 MEDLINE on STN

97070512. PubMed ID: 8913438. **Pharmacokinetic** analysis of protein-**conjugated** doxorubicin (DXR) and its degraded adducts in DXR-sensitive and -resistant rat hepatoma cells. Takahashi N; Asakura T; Ohkawa K. (Department of Biochemistry (I), Jikei University School of Medicine, Tokyo, Japan.) Anti-cancer drugs, **(1996 Aug)** 7 (6) 687-96. Journal code: 9100823. ISSN: 0959-4973. Pub. country: ENGLAND: United Kingdom. Language: English.

AB After treatment of AH66DR cells with the multidrug resistance (MDR) phenotype with bovine **serum albumin** (BSA)-**conjugated** [14C]doxorubicin (DXR), accumulation of the drug in the secondary lysosomal fraction **increased** as a function of time up to 24 h without any significant increase of the drug in other organellae. By contrast, AH66P cells showed a marked increase in accumulation of the drug in the mitochondrial fraction, and a moderate increase in the lysosomal and nuclear fractions. The intracellular degradation of the internalized **conjugate** was assessed by HPLC gel filtration as molecular change of the drug. The initial molecular mass (M(r)) of BSA-**conjugated** [14C]DXR was estimated to be 70 kDa; however, the secondary lysosomal fraction contained mainly three peaks of [14C]compounds ranging from 3 to 70 kDa. The [14C]compound extracted from the nuclear and mitochondrial fractions showed only one peak, which was estimated to be smaller than 2 kDa. By contrast, the cytosolic fraction contained mainly two peaks of [14C]compounds, which were smaller than 2 kDa and larger than 500 kDa. These results indicated that the intracellular distribution of the administered drug, based probably on the drug-traffic mechanism in the cells, was quite different between the two cell lines, but some of the biochemical characteristics of the degraded compounds from each subcellular fraction were similar because the degradation processes in each fraction might be almost identical. The possibility of lysosomal degradation of the protein-**conjugated** DXR leading to expression of cytotoxicity was also confirmed by the fact that only lysosomal digestable poly-L-lysine-**conjugated** DXR exhibited dose-dependent cytotoxicity against both cell lines in marked contrast to the cells treated with poly-D-lysine-**conjugated** DXR. It was concluded that lysosomal breakdown of protein-**conjugated** DXR, which had been taken up by endocytosis, and the liberation of the degraded active adducts of the **conjugate** without efflux by the MDR pump mechanism must be an essential stage in the development of the cytotoxicity against tumor cells with or without the MDR phenotype.

L19 ANSWER 3 OF 9 MEDLINE on STN

96263330. PubMed ID: 8786961. Prolonged circulation of recombinant human granulocyte-colony stimulating factor by covalent linkage to albumin

through a heterobifunctional polyethylene glycol. Paige A G; Whitcomb K L; Liu J; Kinstler O. (Department of Pharmaceutics and Drug Delivery, Amgen Inc., Thousand Oaks, California 91320, USA.) Pharmaceutical research, **(1995 Dec)** 12 (12) 1883-8. Journal code: 8406521.. ISSN: 0724-8741. Pub. country: United States. Language: English.

AB PURPOSE: Recombinant human granulocyte-colony stimulating factor (rhG-CSF) was covalently **conjugated** to both rat and human **serum albumin** (RSA and HSA respectively) to increases the circulating half life (t1/2) of rhG-CSF. METHODS: **Conjugates** of RSA (MW 67,000) and HSA (MW 66,000) were prepared by linking the two proteins through a heterobifunctional maleimido-carboxyl polyethylene glycol (PEG) and were tested in the rat. The **conjugates** were injected intravenously (IV) at the equivalent dose of 50 micrograms/kg of rhG-CSF, and white blood cell (WBC) counts and plasma concentrations of drug were determined. A comparison of **pharmacokinetic** parameters was made between rhG-CSF, the **conjugates** RSA-PEG-rhG-CSF and HSA-PEG-rhG-CSF, and a non-covalent mixture of rhG-CSF and HSA. RESULTS: The **albumin**-rhG-CSF **conjugates** are eliminated more slowly from the circulation. The clearance values are reduced from 0.839 +/- 0.121 ml/min/kg for rhG-CSF to 0.172 +/- 0.013 ml/min/kg for RSA-PEG-rhG-CSF and 0.141 +/- 0.005 ml/min/kg for HSA-PEG-rhG-CSF. WBC counts **increased** in both absolute number and duration as compared to rhG-CSF alone. The **albumin** rhG-CSF **conjugates** had **enhanced** serum stability relative to free rhG-CSF. The rate of degradation of the **albumin conjugates** incubated in rat serum at 37 degrees C decreased five fold. CONCLUSIONS: The results from the study show that specific **conjugation** of rhG-CSF to **albumin** decreases plasma clearance in vivo, causes **increased** WBC response, and increases serum stability as compared to free rhG-CSF.

L19 ANSWER 4 OF 9 MEDLINE on STN

96210093. PubMed ID: 8627575. **Pharmacokinetic** properties of several novel oligonucleotide analogs in mice. Crooke S T; Graham M J; Zuckerman J E; Brooks D; Conklin B S; Cummins L L; Greig M J; Guinosso C J; Kornbrust D; Manoharan M; Sasmor H M; Schleich T; Tivel K L; Griffey R H. (Isis Pharmaceuticals, Inc., Carlsbad, California, USA.) Journal of pharmacology and experimental therapeutics, **(1996 May)** 277 (2) 923-37. Journal code: 0376362. ISSN: 0022-3565. Pub. country: United States. Language: English.

AB Biophysical and **pharmacokinetic** properties of five analogs of ISIS 3082, a 20-mer phosphorothioate oligodeoxynucleotide that inhibits the expression of mouse intercellular adhesion molecule 1, were evaluated. Compared to the parent compound, ISIS 3082, the 2'-propoxy modified phosphodiester, ISIS 9044 and the 2'-propoxy phosphorothioate, ISIS 9045, had **greater** affinity for complementary RNA and were more lipophilic. A chimeric oligonucleotide comprised of 2'-propoxy diester wings and a phosphorothioate deoxy center (ISIS 9046) had equal affinity. It was also more lipophilic than ISIS 3082, but less so than the other 2'-propoxy modified analogs. The two analogs with 5'-lipophilic **conjugates**, ISIS 9047 (5'-octadecylamine) and ISIS 8005 (5'-(2'-O-hexylamino-carbonyl-oxycholesterol) were more lipophilic than ISIS 3082 (3- and 7-fold, respectively) but had similar affinity for complementary RNA. Binding of ISIS 3082 to bovine **serum albumin** was salt-dependent and, at physiological concentration (320 mOsmol), the dissociation constant (Kd) was 140 microM. Similarly, the 2'-propoxy phosphodiester, ISIS 9044, displayed salt-dependent bovine **serum albumin** binding, but not binding was measurable at physiological salt conditions. In contrast, the more lipophilic phosphorothioate analogs displayed much higher affinity to bovine **serum albumin** at 320 mOsmol than ISIS 3082. After bolus injection to mice, the initial volumes of distribution of the more lipophilic phosphorothioate analogs, ISIS 9045, ISIS 9047 and ISIS 8005, were less and the initial clearance from plasma was slower than ISIS 3082. The pharmacokinetics of the other analogs was similar to ISIS 3082. Distribution of ISIS 3082 into peripheral tissues was similar to that reported for other phosphorothioates with liver and kidney accumulating the highest fraction of the dose. The only modification to markedly influence distribution was the very lipophilic cholesterol **conjugate** (ISIS 8005), which **increased** substantially the fraction of the dose accumulated by the liver. Little intact drug was found in urine or feces for any analog, and the patterns of metabolites suggested that for all analogs the principal metabolic pathway was due to 3'-exonuclease activity. The metabolism of ISIS 3082 was similar to that reported for other phosphorothioates. After 2 hr, most of the radioactivity in plasma represented metabolites but, in tissues, intact ISIS 3082 was present for much longer periods of time and metabolites accumulated more slowly. The 24-hr exposure to ISIS 3082 of liver and kidney was 20.7 and 67.9 microM/hr, respectively. The rates of metabolism in plasma, liver and kidney of the two 5'-**conjugates**, ISIS 9047 and ISIS 8005, were similar to ISIS 3082, as was the pattern of metabolism. The rate of metabolism of ISIS 9044 (2'-propoxy phosphodiester oligonucleotide) was much more rapid

in liver and plasma, but surprisingly much slower in the kidney. ISIS 9045 (full 2-propoxy phosphorothioate) was much more stable than ISIS in all tissues, the **enhanced** stability of ISIS 9045 resulted in **increased** exposure of liver and kidney to the drug, whereas the exposure of the liver to the two more lipophilic analogs, ISIS 9047 and ISIS 8005, was **greater** because a higher fraction of the dose was distributed to the liver. The exposure of the kidney to ISIS 9044 was also **greater** than that to ISIS 3082 due to the surprising stability of the drug in the kidney.

L19 ANSWER 5 OF 9 MEDLINE on STN

93221553. PubMed ID: 8466542. Hepatic and intrahepatic targeting of an anti-inflammatory agent with human **serum albumin** and neoglycoproteins as carrier molecules. Franssen E J; Jansen R W; Vaalburg M; Meijer D K. (University Hospital, Department of Nuclear Medicine, The Netherlands.) Biochemical pharmacology, **(1993 Mar 24)** 45 (6) 1215-26. Journal code: 0101032. ISSN: 0006-2952. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The anti-inflammatory agent naproxen (Nap) was covalently coupled to human **serum albumin** (HSA) and to the neoglycoproteins, galactose and mannose terminated HSA, to deliver this drug selectively to different cell types of the liver. Disposition of Nap20-HSA was studied in rats and compared to that of equivalent doses of mixtures of uncoupled drug and protein. The liver to kidney ratios of the drug (L/K-Nap) and the protein (L/K-prot.) were **increased**, indicating an improved delivery of both protein and drug to the target site. After injection of 10 micrograms Nap20-HSA the L/K-prot. was **increased** 15.0 +/- 0.21-fold as measured 1 hr after injection. Even after injection of 5 mg of the **conjugate**, the L/K-prot. was **enhanced** 5.6 +/- 0.34-fold and the L/K-Nap 4.6 +/- 0.23-fold as measured 1 hr after injection. Immunohistochemical staining of liver slices revealed that the endothelial cells were the main sites for hepatic uptake. Further **pharmacokinetic** studies of Nap20-HSA in isolated perfused rat livers showed a saturable uptake process (Vmax = 2.46 micrograms/min/10.0 g liver and Km = 4.27 x 10(-6) M). The uptake in the liver could be inhibited by various polyanionic probes, indicating the major involvement of a scavenger receptor system in the internalization mechanism of Nap20-HSA. This endothelial uptake via the scavenger receptor system is likely to be related to the **increased** negative charge of the Nap-**albumin conjugate** as was revealed by anion exchange chromatography. Studies in the intact organ and in purified liver lysosomal lysates indicate that after internalization of Nap20-HSA the **conjugate** is proteolytically degraded leading to the formation of the lysine **conjugate** of Nap. This amino acid **conjugate** of Nap was shown in a previous study by us to be equipotent to Nap itself with regard to prostaglandin-E2 synthesis inhibition. A pronounced altered intrahepatic distribution was observed when Nap was coupled to lactosaminated and mannosylated HSA (Lact-HSA and Man-HSA, respectively). Coupling of Nap to Lact27-HSA and Man10-HSA resulted in a major shift in intrahepatic distribution from endothelial cells to the hepatocytes and Kupffer cells, respectively. We conclude that **conjugation** of Nap to HSA itself results in a selective delivery to endothelial cells and that the local proteolysis of the **conjugate** produces an active catabolite. Selective delivery to other cell types of the liver can be achieved by attaching naproxen to neoglycoproteins with an appropriate type and number of sugar groups.

L19 ANSWER 6 OF 9 MEDLINE on STN

93058340. PubMed ID: 1279155. Demonstration of the receptor-mediated hepatic uptake of dextran in mice. Nishikawa M; Yamashita F; Takakura Y; Hashida M; Sezaki H. (Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Japan.) Journal of pharmacy and pharmacology, **(1992 May)** 44 (5) 396-401. Journal code: 0376363. ISSN: 0022-3573. Pub. country: ENGLAND: United Kingdom. Language: English.

AB To establish a rationale of designing a drug targeting system using dextran **conjugation**, the disposition behaviour of dextran itself was investigated in mice. At a high dose (100 mg kg-1), [14C]dextran was retained in the blood circulation for a considerably long period. However, [14C]dextran rapidly disappeared from the plasma and accumulated in the liver (up to 60% of dose in 1 h) after a dose of 1 mg kg-1. Cellular localization of [14C]dextran in the liver following intravenous administration was examined and the contribution of parenchymal cells was demonstrated as well as the case of galactosylated bovine **serum albumin** (Gal-BSA). **Pharmacokinetic** analysis based on a physiological model including Michaelis-Menten type uptake mechanisms revealed that the Michaelis constant Km,1 of [14C]dextran was 100 times **greater** than that of Gal-BSA. Coadministration of Gal-BSA delayed the hepatic uptake of [14C]dextran and the simulation based on the physiological model suggested that [14C]dextran was taken up by the same mechanism as Gal-BSA. These results suggested that dextran **conjugation** of a drug might lead to its

undesirable accumulation in the liver at a low dose and an appropriate modification of dextran, such as carboxymethylation, would be required in such cases.

L19 ANSWER 7 OF 9 MEDLINE on STN

92287997. PubMed ID: 1817672. A new radioimmunoassay for the determination of the angiotensin-converting enzyme inhibitor perindopril and its active metabolite in plasma and urine: advantages of a lysine derivative as immunogen to improve the assay specificity. van den Berg H; Resplandy G; de Bie A T; Floor W; Bertrand M; Arts C J. (TNO-CIVO Toxicology and Nutrition Institute, Department of Clinical Biochemistry, Zeist, The Netherlands.) Journal of pharmaceutical and biomedical analysis, **(1991)** 9 (7) 517-24. Journal code: 8309336. ISSN: 0731-7085. Pub. country: ENGLAND: United Kingdom. Language: English.

AB A new radioimmunoassay (RIA) was developed for the direct measurement of perindoprilate (PT), the active metabolite (diacid) of Perindopril (P), an angiotensin-converting enzyme (ACE) inhibitor. Antibodies were raised in rabbits against the lysine derivative of PT **conjugated** to bovine **serum albumin**. The p-hydroxyphenyl derivative of the lysine analogue was used for preparation of the radioligand by iodination (125I). Cross-reactivities for the glucuronide metabolites of P and PT are low (0.25 and 3.5%, respectively). The theoretical limit of detection is 0.2 nM, the sensitivity attainable with random samples is about 0.5 nM. Within- and between-assay variabilities observed were 4.2-6.7 and 2.8-5.9%, respectively (concentration range 2.1-41.7 nM). Serial dilution of plasma and urine samples showed excellent parallelism (r **greater** than 0.95; P less than 0.001). Recoveries of PT spiked to urine and plasma samples were 90-120%. The prodrug P can be measured in the same sample (plasma/urine) after chromatographic separation on a Dowex AG 1 x 2 anion-exchange column and quantitative alkaline hydrolysis of the P-containing fraction. It is concluded that the specificity and sensitivity of this assay are amply sufficient for **pharmacokinetic** studies and in patient monitoring.

L19 ANSWER 8 OF 9 MEDLINE on STN

88026709. PubMed ID: 2444332. Disposition characteristics of mitomycin C-dextran **conjugate** in normal and tumor-bearing muscles of rabbits. Atsumi R; Endo K; Kakutani T; Takakura Y; Hashida M; Sezaki H. (Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Japan.) Cancer research, **(1987 Nov 1)** 47 (21) 5546-51. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Disposition characteristics of the macromolecular prodrug of mitomycin C (MMC), mitomycin C-dextran **conjugate** (MMC-D), in normal and tumor (VX2 carcinoma)-bearing rabbit thigh muscles were studied using the in situ vascular perfusion technique. Three types of cationic MMC-D (MMC-Dcat) and two types of anionic MMC-D (MMC-Dan) with different carrier molecular weights were used. After bolus arterial injection in normal muscles, 83-96% of injected MMC-D was recovered in the venous outflow regardless of the carrier size or charge, whereas less than 60% of MMC was recovered in the same system. By applying statistical moment analysis to the outflow pattern of these drugs, **pharmacokinetic** parameters representing their disposition characteristics were obtained. Smaller intrinsic clearance (CLint) and distribution volume (V) were noted for MMC-D than for MMC, indicating low extravascular diffusion of MMC-D. In the tumor-bearing muscle, blood contamination from other parts of the body **increased** and a shortage of flow recovery due to the neovascularization of the tumors occurred. The disposition parameters of MMC-Dcat with a molecular weight of 500,000 (T-500) indicated some tissue distribution and sequestration in the tumor preparation. After constant infusion of [14C]MMC-D (T-500) for 4 h, tissue radioactivity concentrations were determined in various tissues. A higher radioactivity was observed in the viable region of the tumor and the lymph node compared with the normal muscle tissue and the necrotic region of the tumors. 131I-Labeled human **serum albumin** also gave similar results. In conclusion, higher tumor localization of antitumor agents may be made possible by the application of macromolecular prodrugs.

L19 ANSWER 9 OF 9 MEDLINE on STN

82260775. PubMed ID: 7049571. A simple high-throughput enzymeimmunoassay for norethisterone (norethindrone). Turkes A; Read G F; Riad-Fahmy D. Contraception, **(1982 May)** 25 (5) 505-14. Journal code: 0234361. ISSN: 0010-7824.
Report No.: PIP-009266; POP-00110145. Pub. country: United States. Language: English.

AB A direct enzymeimmunoassay having the sensitivity required for determining norethisterone concentrations in small aliquots of plasma (10 microliter) has been developed. This assay featured a solid phase antiserum raised against a norethisterone-11 alpha-hemisuccinyl/bovine **serum albumin**

conjugate. The antiserum was coupled to cyanogen bromide-activated magnetisable cellulose, and antibody-bound and free fractions were separated by a simple magnetic device. A norethisterone/horseradish peroxidase **conjugate** was used as the label; o-phenylenediamine/hydrogen peroxide being the substrate for colour development. The results obtained by this direct EIA, which allowed processing of at least 100 samples per day, were compared with those of a well-validated enzymeimmunoassay featuring solvent extraction and centrifugal separation of antibody-bound and free steroid; the results were in excellent agreement (n = 30; r **greater** than 0.99) suggesting the usefulness of the simple high-throughput procedure for processing the large sample numbers generated by field investigations and **pharmacokinetic** studies.

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L3            39 S L2 AND PY<2000
L4         15041 S L1 AND PHARMACOLOG?
L5          1533 S L4 AND (CONJUGAT? OR FUSION OR LINKED)
L6             2 S L5 AND (INCREASED STABILITY OR DELAYED CLEARANCE OR INCREASED
L7            47 S L4 AND (MALEIMID?)
L8            28 S L7 AND PY<2000

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L12           13 S L9 AND (ENHANCED PHARMACOKINETIC)
L13           11 S L12 NOT L10

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L18           86 S L17 AND ALBUMIN/AB
L19            9 S L18 AND CONJUGAT?

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NOTES

- Prior art clearly teaches DP-107 and amino/carboxyl truncations; prior art clearly teaches claimed peptide modifications; nothing patentable here.

- Peptides bearing specific amino acid substitutions may be free of the prior art; need to check other HIV-1 isolates and find out motivation/reason for making them.

- US 09/623,533 has similar issues except they are directed toward DP-178 peptides.

92003197. PubMed ID: 1912331. Carbamoylation of peptides and proteins in vitro by S-(N-methylcarbamoyl)glutathione and S-(N-methylcarbamoyl)cysteine, two electrophilic S-linked conjugates of methyl isocyanate. Pearson P G; Slatter J G; Rashed M S; Han D H; Baillie T A. (Department of Medicinal Chemistry, School of Pharmacy, University of Washington, Seattle 98195.) Chemical research in toxicology, **(1991 Jul-Aug)** 4 (4) 436-44. Journal code: 8807448. ISSN: 0893-228X. Pub.country: United States. Language: English.

AB The reactivity toward peptides and proteins of S-(N-methylcarbamoyl)glutathione (SMG), the glutathione conjugate of methyl isocyanate, and the corresponding cysteine adduct, S-(N-methylcarbamoyl)cysteine (SMC), was investigated with the aid of in vitro model systems. Incubation of SMC or a trideuteriomethyl analogue of SMC with either the reduced or oxidized forms of oxytocin afforded similar mixtures of mono-, bis- and tris-N-methylcarbamoylated peptides. Structure elucidation of the mono and bis adducts by fast atom bombardment tandem mass spectrometry indicated that carbamoylation of oxytocin occurred preferentially at Cys-6 and that Cys-1 and/or Tyr-2 were secondary sites of modification. Upon incubation of S-[N-([14C]methyl)carbamoyl]glutathione (14C-SMG) with native bovine **serum albumin** (BSA), radioactivity became bound covalently to the protein in a time- and concentration-dependent fashion. "Blocking" of the lone **Cys-34** thiol group of BSA in the form of a disulfide prior to exposure of the protein to 14C-SMG failed to decrease significantly the extent or time course of this covalent binding. It is concluded that carbamate thioester conjugates of MIC are reactive, carbamoylating entities which can donate the elements of MIC to nucleophilic functionalities on peptides and proteins. Free thiols appear to be preferred sites for such carbamoylation processes, a phenomenon that may have important toxicological consequences in the pathology of tissue lesions induced by MIC and related isocyanates.

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5977313

1998136036. PubMed ID: 9477169. Preparation, characterization and in vitro efficacy of albumin conjugates of doxorubicin. Kratz F; Beyer U; Collery P; Lechenault F; Cazabat A; Schumacher P; Falken U; Unger C. (Tumor Biology Center, Department of Medical Oncology, Clinical Research, Freiburg, Federal Republic of Germany.) Biological & pharmaceutical bulletin, **(1998 Jan)** 21 (1) 56-61. Journal code: 9311984. ISSN: 0918-6158. Pub. country: Japan. Language: English.\

AB One strategy for improving the antitumor selectivity and toxicity profile of antitumor agents is to design drug carrier systems with suitable transport proteins. Thus, four **maleimide** derivatives of doxorubicin were bound to thiolated human **serum albumin** which differed in the stability of the chemical link between drug and spacer. In the **maleimide** derivatives, 3-**maleimidobenzoic** or 4-**maleimidophenylacetic** acid was bound to the 3'-amino position of doxorubicin through a benzoyl or phenylacetyl amide bond and 3-**maleimidobenzoic** acid hydrazide or 4-**maleimidophenylacetic** acid hydrazide was bound to the 13-keto position through a benzoyl hydrazone or phenylacetyl hydrazone bond. The acid-sensitive albumin conjugates prepared with the carboxylic hydrazone doxorubicin derivatives exhibited an inhibitory efficacy in the MDA-MB-468 breast cancer cell line and U937 leukemia cell line comparable with that of the free drug (using the BrdU-(5-bromo-2'-deoxyuridine)-incorporation assay and tritiated thymidine incorporation assay respectively, IC50 approximately 0.1-1 microM) whereas conjugates with the amide derivatives showed no or only marginal activity. These results demonstrate that antiproliferative activity depends on the nature of the chemical bond between doxorubicin and carrier protein. Acid-sensitive albumin conjugates are suitable candidates for further in vitro and in vivo assessment.

96263330. PubMed ID: 8786961. Prolonged circulation of recombinant human granulocyte-colony stimulating factor by covalent linkage to albumin through a heterobifunctional polyethylene glycol. Paige A G; Whitcomb K L; Liu J; Kinstler O. (Department of Pharmaceutics and Drug Delivery, Amgen Inc., Thousand Oaks, California 91320, USA.) Pharmaceutical research, **(1995 Dec)** 12 (12) 1883-8. Journal code: 8406521. ISSN: 0724-8741. Pub. country: United States. Language: English.

AB PURPOSE: Recombinant human granulocyte-colony stimulating factor (rhG-CSF) was covalently **conjugated** to both rat and human **serum albumin** (RSA and HSA respectively) to increases the circulating half life (t1/2) of rhG-CSF. METHODS: **Conjugates** of RSA (MW 67,000) and HSA (MW 66,000) were prepared by linking the two proteins through a heterobifunctional maleimido-carboxyl polyethylene glycol (PEG) and were tested in the rat. The **conjugates** were injected intravenously (IV) at the equivalent dose of 50 micrograms/kg of rhG-CSF, and white blood cell (WBC) counts and plasma concentrations of drug were determined. A comparison of **pharmacokinetic** parameters was made between rhG-CSF, the **conjugates** RSA-PEG-rhG-CSF and HSA-PEG-rhG-CSF,

and a non-covalent mixture of rhG-CSF and HSA. RESULTS: **The albumin-rhG-CSF conjugates are eliminated more slowly from the circulation.** The clearance values are reduced from 0.839 +/- 0.121 ml/min/kg for rhG-CSF to 0.172 +/- 0.013 ml/min/kg for RSA-PEG-rhG-CSF and 0.141 +/- 0.005 ml/min/kg for HSA-PEG-rhG-CSF. WBC counts **increased** in both absolute number and duration as compared to rhG-CSF alone. **The albumin rhG-CSF conjugates had enhanced serum stability relative to free rhG-CSF.** The rate of degradation of the **albumin conjugates** incubated in rat serum at 37 degrees C decreased five fold. CONCLUSIONS: **The results from the study show that specific conjugation of rhG-CSF to albumin decreases plasma clearance in vivo,** causes **increased** WBC response, **and increases serum stability as compared to free rhG-CSF.**

1, 4, 6, 19, 21, 31, 36, 38, 39

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claim 1 (currently amended): A modified anti-viral peptide comprising a peptide having been modified to contain a ~~succinimidyl or~~ maleimide containing group which is reactive with ~~amino groups, hydroxyl groups, or thiol groups on blood components~~ cysteine 34 of serum albumin to form a stable covalent bonds, said peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, and SEQ ID NO:545, said sequence exhibiting an anti-viral activity against human immunodeficiency virus (HIV) wherein said peptide is covalently bonded to cysteine 34 of serum albumin, wherein the ratio of peptide to serum albumin is 1:1.

Claim 2 (canceled)

Claim 3 (canceled)

Claim 4 (previously presented): The modified peptide of claim 1 wherein said peptide is DP 107 and comprises the amino acid sequence of SEQ ID NO:2.

Claim 5 (canceled)

Claim 6 (previously presented): The modified peptide of claim 1 wherein said peptide is selected from the group consisting of SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, and SEQ ID NO:545.

Claims 7-18 (canceled)

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Claim 19 (currently amended): A composition for use in the prevention and/or treatment of acquired immune deficiency syndrome (AIDS) comprising, in a physiologically acceptable medium, a modified peptide including a peptide having been modified to contain a ~~succinimidyl or~~ maleimide containing group which is reactive with ~~amino groups, hydroxyl groups, or thiol groups on blood components~~ cysteine 34 of serum albumin to form a stable covalent bonds, said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, and SEQ ID NO:545, said sequence exhibiting an anti-viral activity against human immunodeficiency virus (HIV) wherein said peptide is covalently bonded to cysteine 34 of serum albumin, wherein the ratio of peptide to serum albumin is 1:1.

Claim 20 (canceled)

Claim 21 (previously presented): The composition of claim 20 wherein said peptide is DP 107 comprising the amino acid sequence of SEQ ID NO:2.

Claims 22-30 (canceled)

Claim 31 (previously presented): The composition of claim 19 wherein said peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, and SEQ ID NO:545.

Claims 32-35 (canceled)

Claim 36 (previously presented): A composition comprising the modified anti-viral peptide of claim 1 in a physiologically acceptable medium.

Claim 37 (canceled)

Claim 38 (previously presented): The composition of claim 36 wherein said modified anti-viral peptide comprises the amino acid sequence of SEQ ID NO:2.

Claim 39 (previously presented): The composition of claim 36 wherein said modified anti-viral peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO:147, SEQ ID NO:148, SEQ ID NO:149, SEQ ID NO:179, SEQ ID NO:180, SEQ ID NO:181, SEQ ID NO:542, SEQ ID NO:543, SEQ ID NO:544, and SEQ ID NO:545.

Claim 40-55 (canceled)